2019

Effects of water availability, longer photoperiod, and elevated [CO2] on the potential of the northward migration in trembling aspen (populus tremuloides michx.)

Inoue, Sahari

Effects of water availability, longer photoperiod, and elevated [CO_2] on the potential of the northward migration in trembling aspen (*populus tremuloides* michx.)

By

Sahari Inoue

A Dissertation Submitted in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy in Forest Sciences

Department of Natural Resources Management

Lakehead University,

Thunder Bay, Ontario, Canada,

August 2019

Abstract

Based on climate envelope models, boreal tree species may migrate to higher latitudes (approximately 10˚N toward the north) in response to rapid climate changes by the end of this century. Multiple factors will likely influence the success of the migration. However, the interactive impacts of these factors are poorly understood. The primary objective of this thesis was to investigate the interactive effect of soil moisture, photoperiod and [CO_2] on the physiology, morphology, and phenology of trembling aspen (*Populus tremuloides* Michx.) in a context of northward migration. Seedlings were exposed to two [CO_2] (AC: 400 vs. EC: 1000 μmol mol^{-1}), four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58°N latitudes and two soil moisture regimes (high soil moisture (HSM) vs. low soil moisture (LSM)) for two growing seasons in environment-controlled greenhouses. I assessed the responses of a suite of morphological, physiological and phenological responses. I found that the longest photoperiod regime (corresponding to 58˚N) had the greatest height growth and total biomass at the first growing season, but cavitation resistance significantly decreases in photoperiod at higher latitude under EC. The results suggest that when migrating to higher latitudes, trembling aspen may grow faster but will become less resistant to drought and more prone to hydraulic failure during a drought spell. There were also significant interactive effects of photoperiod, [CO_2] and soil moisture on the tree physiology. Both stomatal conductance (g_s) and its reduction in response to LSM declined with increasing photoperiod, which can have significant implications for the impact of soil moisture on the tree northward migration. Surprisingly, LSM resulted in an upregulation of photosynthesis as indicated by increases in the maximum rate of Rubisco carboxylation and maximum rate of electron transport for RuBP regeneration, and the magnitude of the upregulation increased with increasing photoperiod. The photosynthetic upregulation was accompanied by an increase in biomass allocation to roots and a reduction in g_s under LSM. LSM increased the instantaneous water-use efficiency (iWUE), and the increase was greater under EC. While LSM increased photosynthesis in both AC and EC in July, the increase was significant under EC in August. EC increased iWUE in both soil moisture treatments in July, but the increase was significant only under LSM in August. Furthermore, EC enhanced net photosynthetic rate (P_n) only under LSM but not HSM. I also found that the significant interactive effects of photoperiod, [CO_2] and soil moisture on phenology. The two longer

photoperiods at 55 and 58˚N latitude significantly delayed growth cessation, bud set and the development of cold hardiness in the fall, particularly under the EC treatment. Although the longest photoperiod resulted in the highest height growth, photoperiod did not have a significant impact on diameter growth or biomass in the second growing season. LSM also delayed phenological events in the fall, but EC delayed bud break in the spring. The significant interactive effects of photoperiod, [CO_2] and soil moisture on phenology, cold hardiness, growth and the length of the growing season indicate the complex nature of latitudinal tree migration that has been predicated under the scenario of climate change. The results of this thesis should advance our understanding of how soil moisture, photoperiod and [CO_2] may interact each other and influence the northward migration of trembling aspen and should also have implications for the potential responses of other boreal tree species, particularly deciduous species. The results of this thesis indicate the importance of understanding interactions of multiple factors in affecting the potential migration of tree species and suggest that [CO_2], photoperiod, and soil moisture should be considered in assessing the potential northward migration of boreal trees in general or human-assisted migration as a response strategy to climate change.

Key-words: Climate change; tree migration; morphology; cavitation resistance; gas exchange; phenology; [CO_2]; soil moisture; photoperiod; *Populus tremuloides* Michx.

Table of contents

List of tables

Table of figures

Abbreviations

AC – Ambient CO_2 concentration

ANOVA – Analysis of variance

C_i/C_a – intercellular to ambient CO_2 concentration

DF – Degree of freedom

DOY – Day of year

DW – Distilled water

EC – Elevated CO_2 concentration

K_{max} – The maximum conductivity of the xylem segment was measured

K_{smax} – The maximum specific hydraulic conductivity

P_n – Net photosynthetic rate

g_s – Stomatal conductance

HSM – High soil moisture

iWUE – Photosynthetic water use efficiency

J_{max} – Maximum rate of photosynthetic electron transport

J_{max}/V_{cmax} – Maximum rate of carboxylation to maximum rate of photosynthetic electron transport

LA – Leaf area

LMR – Leaf mass ratio

LSM – Low soil moisture

LT_{50} – lethal temperature for causing 50% electrolyte leakage

N – Sample number

PAR – photosynthetically active radiation

PLC – The percentage loss of conductivity

RCD – Seedling root collar diameter

REL – Relative electrolyte leakage

RLA – Root length to leaf area ratio

RMR – Root mass ratio

RSR – Root shoot ratio

SLA – Specific leaf area

SMR – Stem mass ratio

SRA – Specific root surface area

SRL – Specific root length

SRR – Shoot to root ratio

V_{cmax} – Maximum rate of carboxylation

Acknowledgments

I am tremendously grateful to all of those who have encouraged and helped me throughout my pursuit of a Doctoral degree.

First and foremost, I wish to express my deepest appreciation to my supervisor, Dr. Qing-Lai Dang, for his extensive guidance and mentoring, and to the members of my supervisory committee, Dr. Rongzhou Man and Dr. David Law, for their feedback over the years, as well as to the external examiners, Dr. Kevin Crowe and Dr. Danielle Way.

I must also thank the members of Eco-physiology lab, especially Binyam Tedla who have been the best colleague and friend, and we shared so many unforgettable moments with countless laughs. I also wish to thank Ms. Joan Lee, former Greenhouse Manager, and Ms. Keri Pidgen, the current Greenhouse Manager of Lakehead University for their logistic support and other operational assistance during the experiments. I would also thank Eva Scollie and Jenn Manion for administrative assistance. I thank the Lakehead University Soils Testing Laboratory for assistance on laboratory works.

I am also grateful to Dr. Hideto Hamada at Sapporo University, who always encourage me with the warmest smile. My long journey started when I met you 10 years ago, and it still goes on.

Lastly but not at all least, I am unconditionally indebted to my family and friends for all their support. Special thanks go to my parents, Masashi and Mitsuko and grandmother, Toyoko, for being always there for me. Other special thanks go to my friends, especially Nozomi Watanabe and Darek Gondor, who encourage and help me always.

This Ph.D. thesis would not have been possible without financial support from the NSERC Discovery grant to Dr. Qing-Lai Dang, Ontario Trillium Scholarship, and Lakehead University Graduate Assistantships.

Co-authorship statement

I have chosen the paper format for this thesis to facilitate the publication of the results in peer-reviewed journals.

Chapters 2 of this dissertation have already been published as Inoue S, Dang Q-L, Man R, Tedla B (2019) Northward migration will increase growth but reduce resistance to drought-induced xylem cavitation in trembling aspen. Botany. doi.org/10.1139/cjb-2019-0099. I was first author for this publication, and the co-authors were Qing-Lai Dang (QLD), Rongzhou Man (RM) and Binyam Tedla (BT). Both QLD and RM contributed to manuscript writing and discussion of ideas. BT contributed to discussion of experimental design and ideas.

Chapter 3 has been submitted to Tree Physiology as Inoue, S., Dang, Q.L., Man, R., and Tedla, B. 2019. Photoperiod and CO_2 elevation influence physiological responses to drought in trembling aspen: implications to climate change-induced migration. I was first author for this publication, and the co-authors were QLD, RM, and BT. QLD contributed to manuscript writing and discussion of ideas. RM contributed to manuscript writing. BT contributed to discussion of experimental design and ideas.

Chapter 4 will be submitted to Plant Cell and Environment as Inoue, S., Dang, Q.L., Man, R., and Tedla, B. 2019. Photoperiod, [CO_2] and soil moisture interactively affect the phenology and growing season length of trembling aspen: a perspective for climate change-induced tree migration. I was first author for this publication, and the co-authors were QLD, RM, and BT. Both QLD and RM contributed to manuscript writing and discussion of ideas. BT contributed to discussion of experimental design and ideas.

1 Chapter 1: General introduction

1.1 Background

Since the Industrial Revolution, there has been a rapid rise in atmospheric CO_2 concentrations ($[CO_2]$), which are predicted to reach approximately 1000 μmol mol^{-1} by 2100 (Keeling et al. 1995, Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014), leading to increases in the annual mean temperature and changes in precipitation patterns (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). The projected future changes in air temperature and precipitation patterns (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014) are expected to affect tree growth and their distribution. Based on atmosphere-ocean General Circulation Models (GCMs), the climate habitat of 130 major North American tree species was estimated by McKenney et al. (2007) and projected to shift 10 degrees toward the north by the end of the century, which is approximately 12000 meters per year of movement. However, the rate of climate change is faster than the capability of tree species migration (Pakeman, 2001). To fill the gap between the rate of climate change and the capability of tree species migration and to alleviate the impact of climate change, human-assisted migration to a suitable climate environment became considered as one of the options for the future (Pedlar et al. 2011, Ste-Marie et al. 2011). Either by natural or human-assisted migration, moving toward the north, however, would expose tree species to many other environmental factors besides climate that affect tree performance, e.g., soil moisture conditions and photoperiod. These factors, coupled with elevated $[CO_2]$, may change tree performance by altering tree responses, including physiology, morphology, and phenology.

Trembling aspen (*Populus tremuloides* Michx.) is the most abundant deciduous tree species in North America including the Canadian boreal forest, a commercially important source of wood

fiber, and has great cultural values for indigenous people (Burns et al. 1990, Peterson and Peterson 1992, Richardson et al. 2007). Trembling aspen provides habitat for wildlife, and is thus an important species for biodiversity (Burns et al. 1990, Perala 1990, Peterson and Peterson 1992). It grows on many soil types, but is known to be extremely vulnerable to water stress (Peterson and Peterson 1992). In past decades, severe droughts coupled with other factors such as insects and pathogens resulted in large areas of decline and die-back of trembling aspen in west-central Canada (Hogg, Brandt, & Michaelian, 2008; Marchetti, Worrall, & Eager, 2011; Worrall et al., 2013). The increase in dieback and mortality of trembling aspen is particularly concerning since more frequent and intense drier soil condition, and thus lower water availability to plants have been predicted for many parts of the boreal forests (Lemprière et al. 2008, Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). Further, during northward migration, trees will be exposed to different photoperiod regimes, which would have a great impact on photoperiod-sensitive tree species, including *Populus* species (Pauley and Perry 1954, Keskitalo et al. 2005). Notably, a shortening day-length triggers the initiation of autumnal phenological events (Pauley and Perry 1954, Keskitalo et al. 2005), which has an important role for cold acclimation toward the winter. Thus, understanding the effect of different photoperiod regimes due to northward migration of trembling aspen is important, especially when combined with other projected factors, including a higher [CO_2] and drier soil condition.

In the following sections, I will present a literature review of photosynthesis, phenology and plant responses to the effect of soil moisture, photoperiod, and elevated [CO_2], and their interactive effects.

1.1.1 Photosynthesis

Photosynthesis is a chemical process that takes place in chloroplast. The process of CO_2 incorporation into carbohydrate, also known as photosynthetic carbon reduction can be divided into three major processes which are carboxylation, reduction, and regeneration. In the carboxylation process, CO_2 is fixed together with ribulose bisphosphate (RuBP) to give two molecules of a three-carbon compound (3-phosphoglyceric acid (3-PGA)) (Hopkins 1999). The reduction phase uses fuel (ATP and NADPH) produced by light reactions of photosynthesis to convert 3-PGA to triose phosphate (TPs). The light reactions of photosynthesis take place in the thylakoid membranes facilitated by the two photosystems (photosystem II and photosystem I) (Grossman et al. 1995). The two photosystems function in series manner: electrons derived from water by photosystem II, are transported by electron carriers called plastoquinone to a proton pump embedded in the membrane, and plastocyanin to photosystem I, which then high energy electrons are passed to ferredoxin coupled with NADP+ to yield NADPH (Zanetti and Curti 1981). The electron transfer reactions also pump proton into the thylakoid lumen where it produces a proton motive force across the thylakoid membrane that powers adenosine triphosphate (ATP) production (Lambers et al. 2008). Both ATP and NADPH will be consumed in reduction phase in the Calvin cycle to convert 3-PGA to TPs. The regeneration phase, which uses ATP to convert part of the TPs reduced in the previous phase back into RuBP which enables the photosynthetic system uninterrupted fixation of CO_2 (Hopkins 1999).

1.1.2 Phenology

Impact of climate change on phenology have attracted the considerable attention of many researchers since plant phenology is one of the most sensitive traits to climate change and key

adaptive traits shaping tree species distribution in North America (Chuine 2010, Kuster et al. 2014, Way and Montgomery 2015). The growing season of temperate and boreal deciduous trees can be defined as the time between bud break and autumnal leaf senescence. If early spring bud break/late autumn leaf senescence occurs, the growing season becomes longer, and if delayed bud break/ early leaf senescence occurs, the growing season becomes shortened. Maximizing the length of growing season may benefit the tree growth (Beuker 1994), but if trees fail to induce a proper timing of dormancy, it can cause severe frost damage in late spring/early autumn and lack of capacity for sufficient cold hardiness during winter (Murray et al. 1989). In most temperate and boreal plant species, dormancy is induced mainly by the increasing length of night in autumn and low temperatures (Weiser 1970, Jackson 2009, Fracheboud et al. 2009, Soolanayakanahally et al. 2013), resulting in onset of autumnal phenological events, such as growth cessation, formation of winter buds, and leaf senescence (Junttila 1980, Clapham et al. 2001, Ruttink et al. 2007, Fracheboud et al. 2009). These phenological events are generally related to the adaption of the species to the local environment (Campbell and Sorensen 1973, Junttila 1982, Kozlowski and Pallardy 2002). Spring phenology such as bud break and leaf unfolding, on the other hand, is largely driven by a function of temperature in most temperate and boreal tree species. Chilling temperature in winter and short photoperiod delay bud break and prevent frost damage, whereas rising temperatures induce bud break (Campbell and Sugano 1979, Aitken and Hannerz 2001, Kozlowski and Pallardy 2002, Howe et al. 2003). Since plant phenological traits, especially in autumn, are genetically adapted to the local environment (Junttila and Nilsen 1993), when trees are grown under common garden experiment, many phenological traits show strong genetic differentiation and clinal variation along the latitudinal gradient (Howe et al. 2003, Savolainen et al. 2007).

1.1.3 Plant responses to low water availability

Since almost every plant process is influenced directly or indirectly by water supply (Kramer and Boyer 1995), water availability is an essential environmental factor that influences tree species distribution in the boreal region (Stephenson 1990). Water availability to plants in most of the forested regions in Canada is expected to decline by 2100 (Lemprière et al. 2008), based on a climatic moisture index (mean annual precipitation minus potential evapotranspiration) (Hogg 1997). Recently, the increase in tree dieback and mortality due to severe water stress has become a critical issue in some parts of North America (Michaelian et al. 2011, Peng et al. 2011, Chen et al. 2017). Under severe water stress conditions, the negative pressure in xylem increases and leads to cavitation, causing an embolism in the xylem conduits. The embolism in the xylem conduit restricts the water supply to leaves and other organs and eventually results in hydraulic failure (Tyree and Zimmermann 2002). Hydraulic failure due to xylem embolism is one of the major mechanisms of drought-induced tree mortality (Allen et al. 2010, McDowell 2011). Plants can undergo physiological and morphological acclimations to maintain xylem water potential above the threshold for hydraulic failure.

Plant physiological acclimation responses to water stress have been well documented, including adjustments at the molecular, biochemical, and physiological levels (Passioura 1996). For short/medium term water deficit, reductions in transpiration rate (E) and stomatal conductance (g_s), and biochemical impairment resulting in a net photosynthetic rate (P_n) reduction are observed (Chaves 1991, Passioura 1996, Flexas et al. 2006). However, recent studies indicate different responses in long-term water stress from those observed in short-term experiments (e.g., Rowland et al. 2015, Zhou et al. 2016). For instance, Zhou et al. (2016) reported the recovery of the maximum rate of Rubisco carboxylation (V_{cmax}) under long-term water stress,

which suggests possible photosynthetic acclimation to long-term water stress. It has also been reported that plants or leaves developed entirely during a long-term water stress have increased nitrogen content and Rubisco concentration, which resulted in photosynthetic up-regulation through increased carboxylation capacity of Rubisco per unit leaf area (Panković et al. 1999, Kitao et al. 2003, Aranda et al. 2005, Kitaoka and Koike 2005). However, since leaf physiological traits are known to be highly plastic, when applied to northward migration, these acclimation responses can be modified by other environmental factors, such as higher [CO_2] and longer photoperiod.

Further, plants also adjust their morphology, including biomass allocation and xylem structure, under long-term water stress. Plants alter their morphology to avoid cavitation induction in their xylem (Sperry 2000). For instance, plants increase their root: shoot ratio to improve water absorption capacity (Kozlowski and Pallardy 2002, Klein et al. 2011). Plants under low water availability tend to have smaller and thicker leaves, and stomatal closure can contribute to minimizing water loss (Kozlowski 1968, Rigling et al. 2010). Increasing xylem resistance to cavitation can also help trees to survive severe water stress (Vilagrosa et al. 2012). In deciduous trees, cavitation resistance is inversely related to the total area of pits in the conduit, which increases with vessel diameter (Hacke and Sperry 2001, Tyree and Zimmermann 2002, Cai and Tyree 2010). Schreiber et al. (2015) found that the vessel diameter of trembling aspen is highly plastic to different environments and varied with summer water availability. Apart from water availability, there are other environmental factors which may affect xylem traits. For instance, elevated [CO_2] enhance tree radial growth and increase vessel diameter in trembling aspen (Kostiainen et al. 2014), and longer photoperiods increase the diameter of xylem tracheid in *Pinus resinosa* (Larson 1962). However, there is a trade-off between hydraulic efficiency and

safety (Fichot et al. 2010). For instance, under drought stress, trees reduce hydraulic conductance to minimize the risk of cavitation at the expense of lowering hydraulic efficiency, which negatively affects growth (Tyree and Zimmermann 2002).

1.1.4 Plant response to photoperiod

Photoperiod is widely considered to be one of the most important key environmental factors for boreal and northern temperate plant species regulating the seasonal changes in, physiology, growth, and phenology. During the period of active growth and winter dormancy, the sequence of physiological and phenological events are synchronized to photoperiod regimes (Weiser 1970, Junttila 1980, Jackson 2009). For instance, Busch et al. (2007) reported that short photoperiod inhibits CO_2 assimilation in *Pinus banksiana L*, even under warm autumn temperatures. Additionally, Bauerle et al. (2012) reported that seasonal changes in photosynthetic capacity (V_{cmax} and the maximum rate of electron transport (J_{max}) is under strong photoperiodic control rather than that of air temperature: though the temperature does not peak at the summer solstice, V_{cmax} peaked immediately after the summer solstice and declined afterward. These studies revealed that plants use photoperiod to regulate seasonal physiological changes, which suggest the importance of photoperiod for boreal and northern temperate plant species. In addition, Benomar et al. (2016) reported that photoperiod at higher latitude enhanced photosynthetic capacity, suggesting the possibility of P_n enhancement. However, in some plant species, an excessive increase in photoperiod reduces the growth rate and photosynthetic efficiency due to the accumulation of starch and soluble sugars in leaves (e.g., Logendra et al. 1990, Shishido et al. 1990, Demers et al. 1998). For morphological responses to photoperiod, Oleksyn et al. (1992) reported that height growth and dry mass were greater under longer photoperiods when 24

populations of Scot pine (*Pinus sylvestris* L.) were grown under conditions emulating changes in photoperiod at 50 and 60˚N.

Similarly, plants use photoperiod signal for the induction of dormancy. Dormancy is induced mainly by the increasing length of night in autumn (short day) and low temperatures (Weiser 1970, Jackson 2009, Fracheboud et al. 2009, Soolanayakanahally et al. 2013), resulting in the onset of autumnal phenological events, such as growth cessation, bud set, and leaf senescence (Junttila 1980, Clapham et al. 2001, Ruttink et al. 2007, Fracheboud et al. 2009). Thus, photoperiodic sensitivity plays an important role in growth and survival for northern tree species. These phenological events are generally related to the adaption of species to the local environment, for instance, northern populations set buds earlier and are hardier than southern populations (Campbell and Sorensen 1973, Junttila 1982, Kozlowski and Pallardy 2002). Because these responses are under strong genetic control, by moving trees north out of their site of origin, trees may experience a mismatch between their phenology and the different photoperiod regimes at the new location. For instance, many common garden experiments have reported that when trees from different latitudinal origins are grown together, the critical photoperiod for phenological traits is expected to reflect underlying latitudinal variation (Pauley and Perry 1954, Ingvarsson et al. 2006, Luquez et al. 2008, Friedman et al. 2011). The plant's response to photoperiod regimes under northward migration may affect their growth and also survival. For instance, growth cessation may be delayed, and while longer growing season would enhance their growth, it increases the risk of frost damage in early autumn (Howe et al. 1995). The frost-damaged stem would prevent a bud break in spring and become susceptible to pest attacks (Ostry et al. 1989), and if trees experience damage annually, their performance such as vigor, height growth, and growth ring width, would be lowered (Zalasky 1976). Further, the

faster photoperiod change during the summer-autumn transition at higher latitudes may jeopardize the proper development of cold hardiness and buds (Bigras et al. 2001). Trees without a proper level of cold hardiness may not survive cold winters (Welling et al. 1997, Li et al. 2003). On the other hand, the delay of autumnal phenology could be beneficial to trees with more opportunities for photosynthesis, which is important for facilitating metabolic processes and nutrient resorption of leaves during leaf senescence (Hoch et al. 2001) and supporting bud break the following spring (Kaelke and Dawson 2005, Regier et al. 2010). Therefore, it is critical to understand how photoperiod regimes at new locations may affect tree growth, dormancy induction, and cold hardiness development.

1.1.5 Plant response to elevated [CO_2]

Elevated [CO_2] affects plant responses in different ways. Since CO_2 is a substrate for photosynthesis, increasing atmospheric [CO_2] will enhance carbon assimilation in C_3 plants (Long et al. 2004, Ainsworth and Rogers 2007). Experimental studies have shown a positive effect of elevated [CO_2] on plant responses (Oberbauer et al. 1985, Ceulemans et al. 1995). For instance, plants generally respond to elevated [CO_2] by photosynthetic stimulation, growth enhancement, and altering biomass allocation (Mott 1990, Long and Drake 1992). The increase in P_n and decreases in g_s improve water use efficiency (*WUE*) (Oberbauer et al. 1985, Tyree and Alexander 1993, Ceulemans et al. 1995, Curtis 1996, Curtis and Wang 1998, Ainsworth and Rogers 2007), which has been shown to ameliorate an impact of water stress to some degree (Wullschleger et al. 2002). However, under long-term exposure of elevated [CO_2], the stimulation of photosynthesis likely become downregulated by a reduction in V_{cmax} through a decrease in Rubisco content (nitrogen investment) (Drake et al. 1997). There are also many studies reporting changes of tree biomass allocation and growth under elevated [CO_2] (Miao et

al. 1992, Lindroth et al. 1993, Overdieck and Forstreuter 1994). Controlled experiments have shown that elevated [CO_2] can increase shoot and root growth (Lavola et al. 2013), leaf area (Overdieck and Forstreuter 1994) and leaf mass (Koch et al. 1986), and total biomass (Lavola et al. 2013).

Elevated [CO_2] can also affect plant autumnal phenology including bud phenology (Murray et al. 1994, Ceulemans et al. 1995a, Jach and Ceulemans 1999), leaf senescence (Tricker et al. 2004, Taylor et al. 2008, Riikonen et al. 2008) and cold hardiness (Tinus et al. 1995, Jach et al. 2001). Elevated [CO_2] has been reported to advance leaf senescence and spring bud break (Jach and Ceulemans 1999), delay leaf senescence in *Populus* species (Tricker et al. 2004, Taylor et al. 2008, Riikonen et al. 2008), or have no effect (Herrick and Thomas 2003). The effect of elevated [CO_2] on spring bud break has been reported with advances (Repo et al. 1996, Karnosky 2003), delays (Murray et al. 1994), and no response (Sigurdsson 2001, Karnosky 2003, Norby et al. 2003).

Several studies on the interactive effects between elevated [CO_2] and other factors on tree responses have been conducted (Miao et al. 1992, Centritto et al. 1999, Zhang and Dang 2006, Danyagri and Dang 2014) and indicated that the results can vary with other environmental factors and also species (Beerling et al. 1996, Saxe et al. 1998a). Thus, it is important to conduct experiments under several factors and understand the interactive effects of these factors on plants responses in the projected new location. For instance, experimental studies have shown a reduction of drought stress under elevated [CO_2] (Wullschleger et al. 2002). C3 plants decrease their stomatal apertures in response to elevated [CO_2], which causes the decline of stomatal conductance (Rey and Jarvis 1998). As a result, water-use efficiency is enhanced (Wullschleger

et al. 2002). However, the interactive effects of soil moisture and elevated [CO_2] depend on species and other environmental factors.

1.2 Aims of this thesis

The objective of my thesis was to investigate the interactive effects of soil moisture, photoperiod and [CO_2] on the physiology, morphology, and phenology of trembling aspen (*Populus tremuloides* Michx) in the context of climate change-induced northward migration. There are several past studies on the interactive effects of elevated [CO_2] and other environmental factors on boreal trees, but the results vary with studies and species (Miao et al. 1992, Centritto et al. 1999, Zhang and Dang 2006, Danyagri and Dang 2014). However, these interactions are still not well understood. A good understanding of such effects, particularly interactions involving photoperiod, is essential for planning latitudinal seed transfer and predicting natural migration of boreal trees in response to climate change. In order to achieve the objective of this thesis, trembling aspen seedlings were exposed to treatments of two [CO_2] (AC 400 and EC 1000 μmol mol^{-1}), four photoperiod regimes corresponding to 48°N (seed origin),52°N, 55°N, and 58°N latitudes, and two soil moisture regimes (high and low) for two growing seasons. I tested the following hypotheses:

- Elevated [CO_2] and longer photoperiod would increase tree growth, and the amount of increase would be reduced by water stress (Chapter 2).
- Water stress would increase xylem cavitation resistance, while elevated [CO_2] and longer photoperiods would have the opposite effect (Chapter 2).
- Longer photoperiods would exacerbate the negative effect of moisture stress on the physiology and growth of trembling aspen (Chapter 3).

- [CO_2] elevation will ameliorate moisture stress and/or overcome the effect of longer photoperiod on biomass partitioning (Chapter 3).
- The changes in photoperiod regime associated with northward migration and elevated [CO_2] would delay autumn phenological events of trembling aspen, resulting in a longer growing season and more growth, and low soil moisture would partially offset the delay in the development of dormancy and cold hardiness (Chapter 4).
- Photoperiod regimes at latitudes north of the seed origin in combination with elevated [CO_2] would expedite bud break in the spring (Chapter 4).

1.3 Reference

Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO_2]: mechanisms and environmental interactions. Plant Cell Environ 30:258–270.

Aitken SN, Hannerz M (2001) Genecology and gene resource management strategies for conifer cold hardiness. In: Bigras FJ, Colombo SJ (eds) Conifer Cold Hardiness. Springer Netherlands, Dordrecht, pp 23–53.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH (Ted), Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running SW, Semerci A, Cobb N (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684.

Aranda I, Castro L, Pardos M, Gil L, Pardos JA (2005) Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quercus suber* L.) seedlings. For Ecol Manag 210:117–129.

Bauerle WL, Oren R, Way DA, Qian SS, Stoy PC, Thornton PE, Bowden JD, Hoffman FM, Reynolds RF (2012) Photoperiodic regulation of the seasonal pattern of photosynthetic capacity and the implications for carbon cycling. Proc Natl Acad Sci 109:8612–8617.

Beerling DJ, Heath J, Woodward FI, Mansfield TA (1996) Drought—CO_2 interactions in trees observations and mechanisms. New Phytol 134:235–242.

Benomar L, Lamhamedi MS, Rainville A, Beaulieu J, Bousquet J, Margolis HA (2016) Genetic adaptation vs. ecophysiological plasticity of photosynthetic-related traits in young *picea glauca* trees along a regional climatic gradient. Front Plant Sci 7.

Beuker E (1994) Adaptation to climatic changes of the timing of bud burst in populations of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. Tree Physiol 14:961–970.

Bigras FJ, Ryyppö A, Lindström A, Stattin E (2001) Cold acclimation and deacclimation of shoots and roots of conifer seedlings. In: Bigras FJ, Colombo SJ (eds) Conifer cold hardiness. Springer Netherlands, pp 57–88.

Burns RM, Honkala BH, Coordinators T (1990) Silvics of north america: volume 2. hardwoods. U S Dep Agric USDA For Serv Agric Handb 654

Busch F, Hüner NPA, Ensminger I (2007) Increased air temperature during simulated autumn conditions does not increase photosynthetic carbon gain but affects the dissipation of

excess energy in seedlings of the evergreen conifer jack pine. Plant Physiol 143:1242–1251.

Cai J, Tyree MT (2010) The impact of vessel size on vulnerability curves: data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx. Plant Cell Environ 33:1059–1069.

Campbell RK, Sorensen FC (1973) Cold-acclimation in seedling douglas-fir related to phenology and provenance. Ecology 54:1148–1151.

Campbell RK, Sugano AI (1979) Genecology of bud-burst phenology in douglas-fir: response to flushing temperature and chilling. Bot Gaz 140:223–231.

Centritto M, Lee HSJ, Jarvis PG (1999) Interactive effects of elevated [CO_2] and drought on cherry (*Prunus avium*) seedlings I. Growth, whole-plant water use efficiency and water loss. New Phytol 141:129–140.

Ceulemans R, Jiang XN, Shao BY (1995) Growth and physiology of one-year old poplar (*Populus*) under elevated atmospheric CO_2 levels. Ann Bot 75:609–617.

Chaves MM (1991) Effects of water deficits on carbon assimilation. J Exp Bot 42:1–16.

Chen L, Huang J-G, Alam SA, Zhai L, Dawson A, Stadt KJ, Comeau PG (2017) Drought causes reduced growth of trembling aspen in western Canada. Glob Change Biol 23:2887–2902.

Chuine I (2010) Why does phenology drive species distribution? Philos Trans R Soc B Biol Sci 365:3149–3160.

Clapham D, Ekberg I, Little CHA, Savolainen O (2001) Molecular biology of conifer frost tolerance and potential applications to tree breeding. In: Bigras FJ, Colombo SJ (eds) Conifer cold hardiness. Springer Netherlands, Dordrecht, pp 187–219.

Curtis PS (1996) A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. Plant Cell Environ 19:127–137.

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113:299–313.

Danyagri G, Dang Q-L (2014) Effects of elevated [CO_2] and soil temperature on photosynthetic responses of mountain maple (*Acer spicatum* L.) seedlings to light. Environ Exp Bot 107:64–70.

Demers D-A, Dorais M, Wien CH, Gosselin A (1998) Effects of supplemental light duration on greenhouse tomato (*Lycopersicon esculentum* Mill.) plants and fruit yields. Sci Hortic 74:295–306.

Drake BG, Gonzàlez-Meler and MA, Long SP (1997) More efficient plants: A consequence of rising atmospheric CO_2? Annu Rev Plant Physiol Plant Mol Biol 48:609–639.

Flexas J, Ribas-Carbó M, Bota J, Galmés J, Henkle M, Martínez-Cañellas S, Medrano H (2006) Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. New Phytol 172:73–82.

Fracheboud Y, Luquez V, Björkén L, Sjödin A, Tuominen H, Jansson S (2009) The control of autumn senescence in European aspen. Plant Physiol 149:1982–1991.

Friedman JM, Roelle JE, Cade BS (2011) Genetic and environmental influences on leaf phenology and cold hardiness of native and introduced riparian trees. Int J Biometeorol 55:775–787.

Grossman AR, Bhaya D, Apt KE, Kehoe DM (1995) Light-harvesting complexes in oxygenic photosynthesis: diversity, Control, and Evolution. Annu Rev Genet 29:231–288.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Perspect Plant Ecol Evol Syst 4:97–115.

Herrick JD, Thomas RB (2003) Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations. Tree Physiol 23:109–118.

Hoch WA, Zeldin EL, McCown BH (2001) Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol 21:1–8.

Hogg EH (1997) Temporal scaling of moisture and the forest-grassland boundary in western Canada. Agric For Meteorol 84:115–122.

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Can J For Res 38:1373–1384.

Hopkins WG (1999) Introduction to plant physiology. Introd Plant Physiol.

Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen TH (2003) From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can J Bot 81:1247–1266.

Howe GT, Hackett WP, Furnier GR, Klevorn RE (1995) Photoperiodic responses of a northern and southern ecotype of black cottonwood. Physiol Plant 93:695–708.

Ingvarsson PK, García MV, Hall D, Luquez V, Jansson S (2006) Clinal variation in phyB2, a candidate gene for day-length-induced growth cessation and bud set, across a latitudinal gradient in European aspen (*Populus tremula*). Genetics 172:1845–1853.

Jach ME, Ceulemans R (1999) Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (*Pinus sylvestris*) seedlings after two years of exposure in the field. Tree Physiol 19:289–300.

Jach ME, Ceulemans R, Murray MB (2001) Impacts of greenhouse gases on the phenology of forest trees. impact carbon dioxide greenhouse gases For Ecosyst CAB Int Wallingford:193–235.

Jackson SD (2009) Plant responses to photoperiod. New Phytol 181:517–531.

Junttila O (1980) Effect of photoperiod and temperature on apical growth cessation in two ecotypes of *Salix* and *Betula*. Physiol Plant 48:347–352.

Junttila O (1982) The cessation of apical growth in latitudinal ecotypes and ecotype crosses of *Salix pentandra* L. J Exp Bot 33:1021–1029.

Junttila O, Nilsen J (1993) Growth and development of northern forest trees as affected by temperature and light. In: Alden JN, Mastrantonio JL, Ødum S (eds) Forest Development in Cold Climates. Springer US, Boston, MA, pp 43–57.

Kaelke CM, Dawson JO (2005) The accretion of nonstructural carbohydrates changes seasonally in *Alnus incana* ssp. rugosa in accord with tissue type, growth, N allocation, and root hypoxia. Symbiosis Rehovot 39:61–66.

Karnosky DF (2003) Impacts of elevated atmospheric CO_2 on forest trees and forest ecosystems: knowledge gaps. Environ Int 29:161–169.

Keeling CD, Whorf TP, Wahlen M, van der Plichtt J (1995) Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. Nature 375:666–670.

Keskitalo J, Bergquist G, Gardeström P, Jansson S (2005) A cellular timetable of autumn senescence. Plant Physiol 139:1635–1648.

Kitao M, Lei TT, Koike T, Tobita H, Maruyama Y (2003) Higher electron transport rate observed at low intercellular CO_2 concentration in long-term drought-acclimated leaves of Japanese mountain birch (*Betula ermanii*). Physiol Plant 118:406–413.

Kitaoka S, Koike T (2005) Seasonal and yearly variations in light use and nitrogen use by seedlings of four deciduous broad-leaved tree species invading larch plantations. Tree Physiol 25:467–475.

Koch KE, Jones PH, Avigne WT, Allen LH (1986) Growth, dry matter partitioning, and diurnal activities of RuBP carboxylase in citrus seedlings maintained at two levels of CO_2. Physiol Plant 67:477–484.

Kostiainen K, Saranpää P, Lundqvist S-O, Kubiske ME, Vapaavuori E (2014) Wood properties of *Populus* and *Betula* in long-term exposure to elevated CO_2 and O_3. Plant Cell Environ 37:1452–1463.

Kozlowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Bot Rev 68:270–334.

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic press.

Kuster TM, Dobbertin M, Günthardt-Goerg MS, Schaub M, Arend M (2014) A phenological timetable of oak growth under experimental drought and air warming. PLOS ONE 9:e89724.

Lambers H, Chapin FSI, Pons TL (2008) Plant physiological ecology. New York: Springer Verlag, c2008.

Larson PR (1962) The indirect effect of photoperiod on tracheid diameter in *Pinus resinosa*. Am J Bot 49:132–137.

Lavola A, Nybakken L, Rousi M, Pusenius J, Petrelius M, Kellomäki S, Julkunen-Tiitto R (2013) Combination treatment of elevated UVB radiation, CO_2 and temperature has little effect on silver birch (*Betula pendula*) growth and phytochemistry. Physiol Plant 149:499–514.

Lemprière TC, Bernier P-Y, Carroll AL, Flannigan MD, Gilsenan RP, McKenney DW, Hogg EH, Pedlar JH, Blain D (2008) The importance of forest sector adaptation to climate change.

Li C, Junttila O, Ernstsen A, Heino P, Palva ET (2003) Photoperiodic control of growth, cold acclimation and dormancy development in silver birch (*Betula pendula*) ecotypes. Physiol Plant 117:206–212.

Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO_2: Productivity, phytochemistry, and insect performance. Ecology 74:763.

Logendra S, Putman JD, Janes HW (1990) The influence of light period on carbon partitioning, translocation and growth in tomato. Sci Hortic 42:75–83.

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: Plants FACE the future. Annu Rev Plant Biol 55:591–628.

Long SP, Drake BG (1992) Chapter 4 - Photosynthetic CO_2 assimilation and rising atmospheric CO_2 concentrations. In: Baker NR, Thomas H (eds) Crop Photosynthesis. Elsevier, Amsterdam, pp 69–103.

Luquez V, Hall D, Albrectsen BR, Karlsson J, Ingvarsson P, Jansson S (2008) Natural phenological variation in aspen (*Populus tremula*): the SwAsp collection. Tree Genet Genomes 4:279–292.

Marchetti SB, Worrall JJ, Eager T (2011) Secondary insects and diseases contribute to sudden aspen decline in southwestern Colorado, USA. Can J For Res 41:2315–2325.

McDowell NG (2011) Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiol 155:1051–1059.

McKenney DW, Pedlar JH, Lawrence K, Campbell K, Hutchinson MF (2007) Potential impacts of climate change on the distribution of north american trees. BioScience 57:939–948.

Miao SL, Wayne PM, Bazzaz FA (1992) Elevated CO_2 differentially alters the responses of coocurring birch and maple seedlings to a moisture gradient. Oecologia 90:300–304.

Michaelian M, Hogg EH, Hall RJ, Arsenault E (2011) Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest: aspen mortality following severe drought. Glob Change Biol 17:2084–2094.

Mott KA (1990) Sensing of atmospheric CO_2 by plants. Plant Cell Environ 13:731–737.

Murray MB, Cannell MGR, Smith RI (1989) Date of budburst of fifteen tree species in Britain following climatic warming. J Appl Ecol:693–700.

Murray MB, Smith RI, Leith ID, Fowler D, Lee HSJ, Friend AD, Jarvis PG (1994) Effects of elevated CO_2, nutrition and climatic warming on bud phenology in Sitka spruce (*Picea sitchensis*) and their impact on the risk of frost damage. Tree Physiol 14:691–706.

Norby RJ, Hartz-Rubin JS, Verbrugge MJ (2003) Phenological responses in maple to experimental atmospheric warming and CO_2 enrichment. Glob Change Biol 9:1792–1801.

Oberbauer SF, Strain BR, Fetcher N (1985) Effect of CO_2-enrichnient on seedling physiology and growth of two tropical tree species. Physiol Plant 65:352–356.

Oleksyn J, Tjoelker MG, Reich PB (1992) Growth and biomass partitioning of populations of European *Pinus sylvestris* L. under simulated 50˚ and 60˚ N daylengths: evidence for photoperiodic ecotypes. New Phytol 120:561–574.

Ostry ME, Wilson LF, McNabb HS (1989) A Guide to Insect, Disease, and Animal Pests of Poplars. United States Department of Agriculture.

Overdieck D, Forstreuter M (1994) Evapotranspiration of beech stands and transpiration of beech leaves subject to atmospheric CO_2 enrichment. Tree Physiol 14:997–1003.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., et al. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Edited ByR.K. Pachauri and L. Meyer. IPCC, Geneva, Switzerland.

Pakeman (2001) Plant migration rates and seed dispersal mechanisms. J Biogeogr 28:795–800.

Panković D, Sakač Z, Kevrešan S, Plesničar M (1999) Acclimation to long-term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. J Exp Bot 50:128–138.

Passioura JB (1996) Drought and drought tolerance. Plant Growth Regul 20:79–83.

Pauley SS, Perry TO (1954) Ecotypic variation of the photoperiodic response in *populus*. J Arnold Arbor 35:167–188.

Pedlar J, McKenney D, Beaulieu J, Colombo S, McLachlan J, O'Neill G (2011) The implementation of assisted migration in Canadian forests. For Chron 87:766–777.

Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X, Zhou X (2011) A drought-induced pervasive increase in tree mortality across Canada's boreal forests. Nat Clim Change 1:467–471.

Perala DA (1990) *Populus tremuloicies* Michx.-Quaking Aspen. Silv N Am Hardwoods Burns RM Honkala BH Eds:555–569.

Peterson EB, Peterson NM (1992) Ecology, management and use of aspen and balsam poplar in the prairie provinces, Canada. Special Report 1.

Regier N, Streb S, Zeeman SC, Frey B (2010) Seasonal changes in starch and sugar content of poplar (*Populus deltoides* × *nigra* cv. *Dorskamp*) and the impact of stem girdling on carbohydrate allocation to roots. Tree Physiol 30:979–987.

Repo T, Hänninen H, Kellomäki S (1996) The effects of long-term elevation of air temperature and CO_2 on the frost hardiness of Scots pine. Plant Cell Environ 19:209–216.

Rey A, Jarvis PG (1998) Long-term photosynthetic acclimation to increased atmospheric CO_2 concentration in young birch (*Betula pendula*) trees. Tree Physiol 18:441–450.

Richardson J, Cooke JEK, Isebrands JG, Thomas BR, Van Rees KCJ (2007) Poplar research in Canada — a historical perspective with a view to the future. Can J Bot 85:1136–1146.

Riikonen J, Kets K, Darbah J, Oksanen E, Sober A, Vapaavuori E, Kubiske ME, Nelson N, Karnosky DF (2008) Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO_2 and O_3. Tree Physiol 28:243–254.

Rowland L, Lobo-do-Vale RL, Christoffersen BO, Melém EA, Kruijt B, Vasconcelos SS, Domingues T, Binks OJ, Oliveira AAR, Metcalfe D, Costa ACL da, Mencuccini M, Meir P (2015) After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration. Glob Change Biol 21:4662–4672.

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390.

Savolainen O, Pyhäjärvi T, Knürr T (2007) Gene flow and local adaptation in trees. Annu Rev Ecol Evol Syst 38:595–619.

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139:395–436.

Schreiber SG, Hacke UG, Hamann A (2015) Variation of xylem vessel diameters across a climate gradient: insight from a reciprocal transplant experiment with a widespread boreal tree. Funct Ecol 29:1392–1401.

Shishido Y, Seyama N, Imada S, Hori Y (1990) Effect of the Photosynthetic Light Period on the Carbon Budget of Young Tomato Leaves. Ann Bot 66:729–735.

Sigurdsson BD (2001) Elevated [CO_2] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. Trees 15:403–413.

Soolanayakanahally RY, Guy RD, Silim SN, Song M (2013) Timing of photoperiodic competency causes phenological mismatch in balsam poplar (*Populus balsamifera* L.). Plant Cell Environ 36:116–127.

Ste-Marie C, A. Nelson E, Dabros A, Bonneau M-E (2011) Assisted migration: Introduction to a multifaceted concept. For Chron 87:724–730.

Stephenson NL (1990) Climatic control of vegetation distribution: The role of the water balance. Am Nat 135:649–670.

Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Gioli B, Calfapietra C, Gielen B, Kubiske ME, Scarascia-Mugnozza GE, Kets K, Long SP, Karnosky DF (2008) Future atmospheric CO_2 leads to delayed autumnal senescence. Glob Change Biol 14:264–275.

Tinus RW (usda, Greer DH, Robinson LA (1995) Effect of high CO_2 on cold acclimation and deacclimation of three conifers. Gen Tech Rep RM USA.

Tricker PJ, Calfapietra C, Kuzminsky E, Puleggi R, Ferris R, Nathoo M, Pleasants LJ, Alston V, Angelis PD, Taylor G (2004) Long-term acclimation of leaf production, development, longevity and quality following 3-year exposure to free-air CO_2 enrichment during canopy closure in *Populus*. New Phytol 162:413–426.

Tyree MT, Alexander JD (1993) Plant water relations and the effects of elevated CO_2: a review and suggestions for future research. Vegetatio 104:47–62.

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap, 2nd edn. Springer-Verlag, Berlin Heidelberg.

Vilagrosa A, Chirino E, Peguero-Pina JJ, Barigah TS, Cochard H, Gil-Pelegrín E (2012) Xylem cavitation and embolism in plants living in water-limited ecosystems. In: Aroca R (ed) Plant responses to drought stress. Springer Berlin Heidelberg, pp 63–109.

Way DA, Montgomery RA (2015) Photoperiod constraints on tree phenology, performance and migration in a warming world. Plant Cell Environ 38:1725–1736.

Weiser CJ (1970) Cold Resistance and Injury in Woody Plants: Knowledge of hardy plant adaptations to freezing stress may help us to reduce winter damage. Science 169:1269–1278.

Welling A, Kaikuranta P, Rinne P (1997) Photoperiodic induction of dormancy and freezing tolerance in *Betula pubescens*. Involvement of ABA and dehydrins. Physiol Plant 100:119–125.

Worrall JJ, Rehfeldt GE, Hamann A, Hogg EH, Marchetti SB, Michaelian M, Gray LK (2013) Recent declines of *Populus tremuloides* in North America linked to climate. For Ecol Manag 299:35–51.

Wullschleger SD, Tschaplinski TJ, Norby RJ (2002b) Plant water relations at elevated CO_2 -- implications for water-limited environments. Plant Cell Environ 25:319–331.

Zalasky H (1976) Frost damage in poplar on the prairies. For Chron 52:61–64.

Zanetti G, Curti B (1981) Interactions between ferredoxin-nadp+ reductase and ferredoxin at different reduction levels of the two proteins. FEBS Lett 129:201–204.

Zhang S, Dang Q-L (2006) Effects of carbon dioxide concentration and nutrition on photosynthetic functions of white birch seedlings. Tree Physiol 26:1457–1467.

Zhou S-X, Medlyn BE, Prentice IC (2016) Long-term water stress leads to acclimation of drought sensitivity of photosynthetic capacity in xeric but not riparian *Eucalyptus* species. Ann Bot 117:133–144.

2 Chapter 2: Northward migration will increase growth but reduce resistance to drought-induced xylem cavitation in trembling aspen

2.1 Abstract

Trees may migrate to higher latitudes in response to climate changes in the future, exposing them to higher [CO_2], new photoperiods, different soil moisture, and other new conditions. The new conditions may influence tree physiology, survival, and growth. This study examined the interactive effects of [CO_2], photoperiod and soil moisture on morphology and cavitation resistance in trembling aspen. One-year-old seedlings were exposed to two [CO_2] (AC 400, EC 1000 µmol mol^{-1}), four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58°N latitudes, and two soil moistures (60%-75% and 13% -20% of field capacity) for one growing season in greenhouses. Seedling growth, leaf size, specific leaf area, biomass allocations and xylem resistance to cavitation (water potentials for 20, 50 and 80% loss of hydraulic conductivity) were assessed. The seedlings under the longest photoperiod regime (58˚N latitude) had greatest height and biomass but smallest specific leaf area. Under EC, however, the longest photoperiod regime significantly reduced xylem resistance to drought-induced cavitation as compared to the photoperiod at 48˚N. The results suggest that when migrating to higher latitudes trembling aspen may grow faster but could become less resistant to drought and more prone to hydraulic failure during a drought spell.

Keywords: Climate change, tree migration, morphology, cavitation resistance.

2.2 Introduction

The projected rises in global temperatures and changes in precipitation patterns will likely induce northward migration of boreal trees by the end of this century (McKenney et al. 2007, 2011). As a consequence, trees will be exposed to a new environment with different photoperiod regimes and higher carbon dioxide concentration ([CO_2]). The effects of photoperiod and [CO_2] alone on trees have been studied (Oleksyn et al. 1992, Norby et al. 2010), but it is unknown how these factors interact in affecting trees in the context of predicted northward migration. It is also predicted that the frequency and severity of water stress will increase in many regions of North America (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). Increased water stresses will likely hinder the northward migration of boreal trees, e.g., trembling aspen (*Populus tremuloides* Michx.). Trembling aspen is widely distributed in the boreal region and has high economic and ecological values, but is very sensitive to water stress (Perala 1990, Peterson and Peterson 1992). Under severe droughts, trembling aspen a good understanding of how trembling aspen may respond to drought stress under such new environmental conditions may be essential for planning latitudinal seed transfer and predicting the natural can have widespread dieback and mortality (Hogg et al. 2008, Michaelian et al. 2011). Therefore, the migration of boreal trees in response to climate change.

Hydraulic failure due to xylem cavitation is a major mechanism of drought-induced tree mortality (McDowell et al. 2008, Allen et al. 2010, Adams et al. 2017). Trees can undergo physiological and/or morphological acclimations to maintain xylem water potential above the threshold for hydraulic failure, e.g., to increase root: shoot ratio (Kozlowski and Pallardy 2002, Klein et al. 2011) and/or stomatal sensitivity to changes in water status, or to have smaller and thicker leaves (Kozlowski 1968, Rigling et al. 2010). Increasing xylem resistance to cavitation

can also help trees to survive severe drought stress (Vilagrosa et al. 2012). In deciduous trees, cavitation resistance is inversely related to the total area of pits in conduit, which increases with vessel diameter (Hacke and Sperry 2001, Tyree and Zimmermann 2002, Cai and Tyree 2010). However, there is a trade-off between hydraulic efficiency and cavitation resistance (Fichot et al. 2010). For instance, trees grown under mild drought stress have more resistant xylem, such as smaller diameter of conduits and lower pit area, which lead to a reduction in hydraulic conductance and the risk of cavitation at the expense of lowering hydraulic efficiency (Tyree and Zimmermann 2002).

The interactions among [CO_2], photoperiod and water availability can influence physiological and morphological characteristics of trees that have a significant impact on the northward migration or assisted seed transfer of boreal trees. For instance, CO_2 elevations increase tree growth and improve water use efficiency (WUE) (Norby et al. 1999, Centritto et al. 2002) and thus mitigate the negative effects of drought stress on growth. Longer photoperiods during the growing season increase shoot: root ratio and enhance leaf development (Oleksyn et al. 1992, Gestel et al. 2005, Abeli et al. 2015), resulting in improved hydraulic efficiency but increased water loss. Further, higher [CO_2] increase vessel diameter in aspen (Kostiainen et al. 2014), and longer photoperiods increase the diameter of xylem tracheid in *Pinus resinosa* (Larson 1962), which may improve hydraulic efficiency but also reduce cavitation resistance. However, there is generally a paucity of information on the interactive effects of [CO_2], photoperiod and soil moisture on the morphological and hydraulic characteristics of boreal trees. For example, Newaz et al. (2017) examined the interactive effect of [CO_2], photoperiod and soil moisture on the hydraulic characteristics of jack pine in the context of northward migration and found significant increase in hydraulic conductivity under higher [CO_2], and cavitation resistance decreased with

increasing photoperiod under elevated [CO_2]. The present study investigated the fore mentioned three-way interaction on the morphology and resistance to cavitation in trembling aspen in the context of climate change-induced northward migration. We hypothesized that elevated [CO_2] and longer photoperiod would increase tree growth and the amount of increase would be reduced by water stress. We further hypothesized that water stress would increase xylem cavitation resistance while elevated [CO_2] and longer photoperiods would have the opposite effect.

2.3 Materials and Methods

2.3.1 Plant materials

Trembling aspen (*Populus tremuloides* Michx.) catkins were collected in June 2016 from 10 free-pollinated trees in Thunder Bay region, Ontario, Canada (48.38°N, 89.25°W). The catkins were air-dried for five days at room temperature, and seeds were extracted. All the seeds were mixed and stored in a sealed glass bottle at -4°C. Seeds were sown in germination trays filled with a mixture of peat moss and vermiculite (1:1 (v/v)) in a greenhouse at the Thunder Bay Campus of Lakehead University. The greenhouse was maintained at 24/14 (±2) °C (day/night) temperature, and the natural photoperiod was prolonged to 16h by using high-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada) which amounted to about 600 $\mu mol \cdot m^{-1} \cdot s^{-1}$ PAR at the canopy level. All trays were kept under ambient [CO_2] and well-watered. The seedlings (n = 96) were individually transplanted to circular pots (12 cm in height, and 12.5 cm and 9.5 cm top and bottom diameter, respectively) filled with a mixture of peat moss and vermiculite (7:3 v/v) 18 days after germination.

2.3.2 Experimental design

The experiment was conducted in the Forest Ecology Complex of Lakehead University, Thunder

Bay campus. The treatments consisted of two [CO_2] (AC 400 vs. EC 1000 µmol mol^{-1}), four photoperiod regimes corresponding 48°N (seed origin),52°N, 55°N, and 58°N latitudes, and two soil moisture regimes (high vs. low). The experiment was a split-split plot design with [CO_2] as the main plots, photoperiods as the sub-plots, and soil moisture level as the sub-sub-plots. Each [CO_2] level had two independent replicates and were randomly assigned to two of the four greenhouses. I used a total 96 seedlings: 3 seedlings per treatment combination (2 levels of CO_2, 4 photoperiods, and 2 soil moisture levels) and 2 replications per combination, $3 \times 2 \times 4 \times 2 \times 2 = 96$).

2.3.3 Treatments and greenhouse environment controls

The environmental conditions in each greenhouse were independently monitored and controlled using the Argus Titan Environment Control System (Argus Controls Systems Ltd., Surrey, BC, Canada). [CO_2] was elevated using the model GEN2E gas CO_2 generators made by Custom Automated Products Inc. (Riverside, California, USA). The photoperiod in each greenhouse was set to the longest of the four treatment levels by supplementing natural light (on cloudy days, and in early mornings and late evenings) using high-pressure sodium lamps (Model LR48877, P.L. Systems, Grimspy, ON, Canada). The shorter photoperiods were obtained by manual shading. A wooden frame was installed for each photoperiod treatment and efforts were made to make sure that all light was blocked with neutral density shading cloth. The light intensity inside the shaded-frame was 0 µmol s^{-1} m^{-2} as measured with a light meter (LI-250, LI-COR Inc., USA) with a quantum sensor (LI-190, LI-COR Inc., USA)). The photoperiod setting was adjusted weekly according to the calculated photoperiod for each of the four latitudes. The soil volumetric water content was monitored using Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK) and kept between 60% and 75% of the field capacity for the high

moisture treatment and between 13% and 20% of the field capacity for the low soil moisture treatment.

The experiment was run from November 1st to February 4th, 2016/17 to emulate the natural growing season from June 7th to September 10th. The temperature and photoperiod were adjusted weekly based on the weekly averages of the past ten years for each of the four latitudes (Environment Canada Weather Records). The daily temperature in each greenhouse was ramped at 6-hour set points (averages of the past ten years). We fertilized seedlings in three phases with different fertilizer formulations: Rapid growth phase (June 7th to June 21st): 100 mg N L^{-1}, 60 mg P L^{-1}, 150 mg K L^{-1}, 80 mg Ca L^{-1}, 40 mg Mg L^{-1} and 60 mg S L^{-1}; Establishment phase (June 22nd to August 30th): 33 mg N L^{-1}, 73 mg P L^{-1} and 55 mg K L^{-1}; Hardening phase (September 1st to the end of the experiment): 33mg N L^{-1}, 44 mg P L^{-1} and 83 mg K L^{-1} (Landis 1989, Jacobs and Landis 2009).

2.3.4 Measurements and assessments

Growth Measurements

Three seedlings were randomly selected from each treatment combination (2 levels of [CO_2], 4 photoperiods, and 2 levels of soil moisture) and 2 replications per combination ($3 \times 2 \times 4 \times 2 \times 2 = 96$ seedlings in total). The randomization was carried out at the time when measurements were taken. The selected seedlings were harvested after height and root collar diameter (RCD) measurement and were separated into leaves, stem, and roots. The leaves from each seedling were scanned to determine total leaf area (LA) and the number of leaves was using the WinFolia software (Regent Instruments Inc, Quebec, Canada). The root system was washed individually. Dry mass components were weighed separately after oven-drying for 48 hours at 80°C and used

for calculations of biomass allocation, specific leaf area (SLA= total leaf area / total leaf dry mass), leaf area ratio (LAR= leaf area/ total dry mass leaf mass ratio), leaf mass ratio (LMR leaf dry mass/ total dry mass), stem mass ratio (SMR= stem dry mass/ total dry mass), root mass ratio (RMR= root dry mass/total dry mass), and root to shoot ratio (RSR= root dry mass/ shoot dry mass).

Xylem Conductivity and Cavitation Resistance Assessments

Three seedlings were randomly selected from each treatment combination as described previously. The randomization was carried out at the time of measurement. The air-injection method (Sperry and Saliendra 1994) using a double-ended pressure chamber (1505D-EXP Pressure Chamber, PMS Instrument Company, Albany, OR, USA) was used to create cavitation in the xylem. Stems were cut into 15cm segments to accommodate long maximum vessel lengths (mean vessel length 1.9cm, between 8 and 15cm of stems; Sperry and Saliendra 1992; Sperry et al. 1994) at 5 cm from root collar under water to avoid embolism in xylem conduits (Wheeler et al. 2013). The bark at the end of stem segment was removed with a razor blade and the xylem was covered with parafilm, which was attached to a flexible tube connected to a solution tank filled with distilled water and 20mM/L of Potassium chloride. The bark in the middle of the segment (5 cm) was removed to ensure air entry into the xylem (Ennajeh et al. 2011). The tank was installed at 0.5m in height. The segment was installed in the doubled-ended pressure chamber. To make the sample segments fully functional (no embolism in the xylem conduits), the segments were flushed at 175 kPa pressure for 20 min to remove all air bubbles. After full saturation, the maximum conductivity of the xylem segment was measured (K_{max}), which was divided by the functional xylem area of each segment to obtain maximum specific hydraulic conductivity (K_{smax}). The hydraulic conductivity was measured according to Sperry et al. (1988).

A gravity-induced flow rate (5 min) and 10kPa-pressure-induced flow rate (5 min) were measured, and the gravity-induced flow rate was subtracted from 10kPa-induced flow rate. The hydraulic conductivity (K_h) was defined as the mass flow rate (K_{max}) through the segment divided by the pressure difference/segment length;

$$(K_h)=\frac{\text{Mass flow rate (mg } s^{-1})}{\text{Pressure difference(MPa)/Segment length (m)}}$$

After getting k_{max}, the segment was pressurized to create cavitation in the xylem. The segment was pressurized with different pressure (0.5, 1.0, 1.5, 1.8, 2.0, 2.5, 3.0, 3.5, and 4.5 MPa) for 10 min. The K_h measurement was initiated after the stem stopped releasing air and continued until the percentage loss of conductivity (*PLC*) was > 95%. The *PLC* was defined as,

$$(PLC)=100\times(1-\frac{K_h}{K_{max}})$$

The relationship between hydraulic conductance and decreasing water potential (-MPa) for each seedling was fitted with a Weibull curve using the "fitplc" function in the fitplc package (Duursma and Choat, 2017) in R software (Version 3.5.0, R Development Core Team 2018). From these curves, the water potential corresponding to 20%, 50% and 80% loss of K_h ($P_{20,}$ $P_{50,}$ and P_{80}) and bootstrapped confidence intervals (CIs; 95%) were calculated. Differences in vulnerability between treatments were deemed significant only if CIs did not overlap.

2.3.5 Data Analysis

All the data were examined graphically for the normality of distribution (probability plots of residuals) and homogeneity of variance (scatter plots) with the R software (Version 3.5.0, R Development Core Team 2018) before being subjected to the analysis of variance (ANOVA). ANOVA was used to test the effects of [CO_2], photoperiod, and soil moisture regimes and their

interactions. When the ANOVA showed a significant ($p \leq 0.05$) photoperiod effect or a significant interaction, Fisher's Least Significant Difference (LSD) post hoc test was used to examine differences among treatment means. There were limitations in pair-wise comparisons in the split-split plot experimental design we used in this study because lower level plots were nested within a higher-level plot. Furthermore, the sample size in this study was very small, increasing the likelihood of real treatment effects being undetected (type II error). Fisher's LSD offers a reasonable control of type II error without compromising the power of the statistical test for split plot and split-split plot designs. All the analyses were performed using R software (Version 3.5.0, R Development Core Team 2018).

2.4 Results

Growth and biomass allocation

The seedlings in the longest photoperiod (58˚N) treatment had significantly higher height growth (110 ± 17.55cm) and smaller specific leaf area (SLA) (363.49 ± 90.81 $cm^2 g^{-1}$) than all other shorter photoperiod treatments (92.19 ± 17.16 cm, 432.63 ± 104.18 $cm^2 g^{-1}$) (Table 1; Figure 1a-right, 2b). Low soil moisture had significantly lower height (92.74 ± 17.70 cm), RCD (7.03 ± 1.14 mm) and leaf size (56.84 ± 13.87 cm^2) than that of high soil moisture treatment (100.30 ± 19.19 cm, 7.98 ± 1.32 mm, 65.33 ± 14.24 cm^2) (Table 1; Figures 1a-left, b, 2a).

The seedlings in the longest photoperiod had significantly greater total biomass (18.93 ± 6.68 g) and leaf biomass (6.49 ± 1.97 g) than those in the shorter photoperiods (15.04±7.28 g, 5.07±2.09 g) (Table 1; Figure 3a-left, b-left). Similarly, the seedlings at low soil moisture were lower in total biomass (13.20 ± 5.35 g), leaf biomass (4.67 ± 1.53 g), stem biomass (5.61 ± 2.44 g), and root biomass (2.39 ± 1.47 g) compared to seedlings in high soil moisture (18.82 ± 7.94 g, 6.18 ±

2.40 g, 8.24 ± 3.47 g, 3.74 ± 2.20 g) (Figure 3a-right, b-right and c). The root biomass difference between low and high soil moisture was significantly greater at the combinations of AC and the photoperiod at 58°N (High:4.87 ± 1.71g, Low: 2.00 ± 0.97g) and EC and the photoperiod at 52°N (High:5.06 ± 3.21g, Low: 1.43 ± 0.97g) (Figure 3d) (significant three-way interaction).

Table 2.1 Summary of ANOVA for the effects of [CO_2] (C), photoperiod (P), soil moisture (SM) and their interactions on height, RCD, leaf size, SLA, total dry weight (DW), leaf DW, stem DW, root DW, leaf mass ratio (LMR), stem mass ratio (SMR), root mass ratio (RMR), and root shoot ratio (RSR). The seedlings were exposed to two levels of [CO_2] (400 vs. 1000 µmol mol^{-1}), four photoperiod regimes (at 48.38°N (seed origin), 51.68°N, 54.98°N, and 58.28°N), and two soil moisture regimes (high (60%-75%) vs. low (13% -20%)). The numbers in bold are significant *p*-values at <0.05.

Variable		C (DF=1)	P (DF=3)	SM (DF=1)	C × P (DF=3)	C × SM (DF=1)	P × SM (DF=3)	C × P × SM (DF=3)
Height	*F*	0.427	13.761	8.875	0.528	0.070	2.314	3.001
	P	0.581	**0.004**	**0.018**	0.679	0.798	0.152	0.095
RCD	*F*	0.605	0.796	28.739	1.213	0.059	0.909	1.203
	P	0.518	0.539	**0.001**	0.383	0.815	0.479	0.369
Leaf size	*F*	0.736	0.553	7.940	1.013	0.044	0.117	0.419
	P	0.481	0.665	**0.023**	0.450	0.838	0.948	0.744
SLA	*F*	0.964	4.933	2.749	1.304	0.518	0.794	1.106
	P	0.430	**0.047**	0.136	0.357	0.492	0.531	0.402
Total DW	*F*	0.966	6.889	37.817	3.115	0.081	1.606	3.332
	P	0.429	**0.023**	**<0.001**	0.110	0.783	0.263	0.077
Leaf DW	*F*	0.485	10.542	38.056	3.627	0.164	2.139	1.699
	P	0.558	**0.008**	**<0.001**	0.084	0.696	0.173	0.244
Stem DW	*F*	1.211	4.440	42.117	1.257	0.157	1.459	3.347
	P	0.386	0.057	**<0.001**	0.370	0.703	0.297	0.076
Root DW	*F*	2.273	4.380	22.737	3.956	0.266	1.021	4.607
	P	0.271	0.059	**0.001**	0.072	0.620	0.433	**0.037**
LMR	*F*	0.480	1.544	12.290	0.685	0.103	0.249	2.547
	P	0.560	0.297	**0.008**	0.593	0.757	0.860	0.129
SMR	*F*	0.160	2.032	8.893	0.245	0.017	0.204	5.557
	P	0.728	0.211	**0.018**	0.862	0.899	0.891	**0.023**
RMR	*F*	0.450	5.339	4.332	2.066	0.043	0.805	4.179
	P	0.571	**0.040**	0.071	0.206	0.841	0.525	**0.047**
RSR	*F*	0.615	5.278	4.221	2.102	0.021	0.784	4.298
	P	0.515	**0.040**	0.074	0.202	0.890	0.536	**0.044**

*DF=degree of freedom

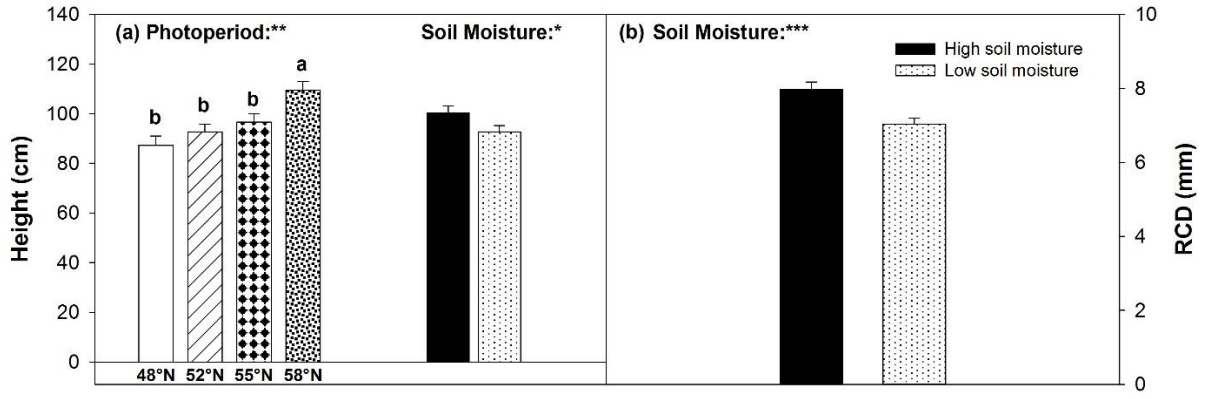

Figure 2.1 Total height (Mean + SE) by four photoperiods (at 48 (seed origin), 52, 55, and 58°N) (a) and two soil moisture (high and low) (b) in trembling aspen seedlings. The Figure (b) represents the mean root collar diameter (RCD) by two soil moisture. The data in Figure (a) were pooled for the 2 levels of [CO_2] and 2 levels of soil moisture treatments because the 3-way interaction was not statistically significant (n = 3 x 2 x 2 x 2 = 24). The sample size for Figure (b) was 48 because the data were pooled for the 2 levels of [CO_2] and 4 levels of photoperiods due to p >0.05 (3 seedlings x 2 replicates x 2 [CO_2] x 4 photoperiods = 48). Different letters above the bars indicate significant differences under Fisher's Least Significant Difference (LSD) post hoc test (p < 0.05). The level of significance is shown (*p <0.05; ** p <0.01; *** p <0.001).

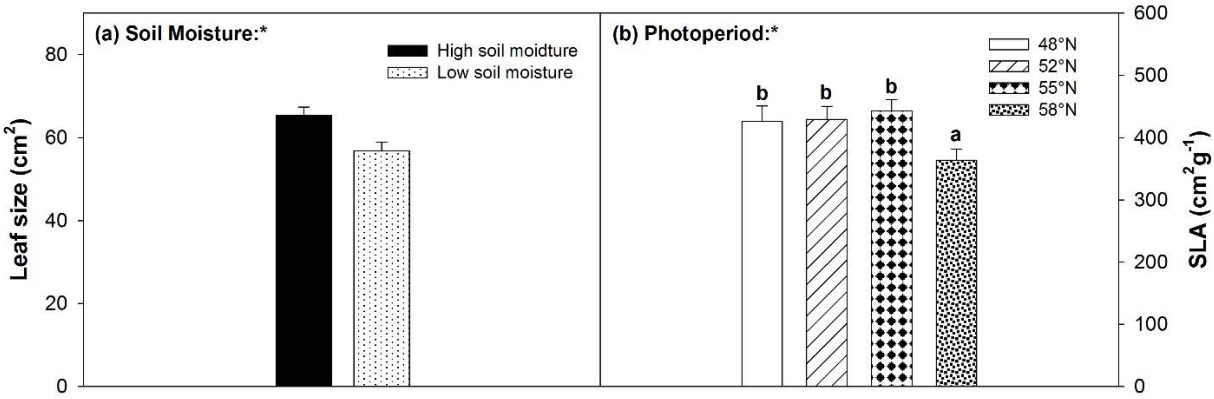

Figure 2.2 Mean leaf size by two soil moisture (high vs. low) (a) and specific leaf area by four photoperiods (at 48 (seed origin), 52, 55, and 58°N) (b) in trembling aspen seedlings (Mean + SE). The data for Figure (a) were pooled for [CO_2] and photoperiod because p> 0.05 for the 3-way interaction (3 seedlings per treatment combination x 2 replications per combination x 2 [CO_2] x 4 photoperiods = 48). Data for Figure (b) were pooled for the 2 [CO_2] and 2 soil moisture treatments (n = 3 x 2 x 2 x 2 = 24). The level of significance is shown (*p <0.05; ** p <0.01; *** p <0.001).

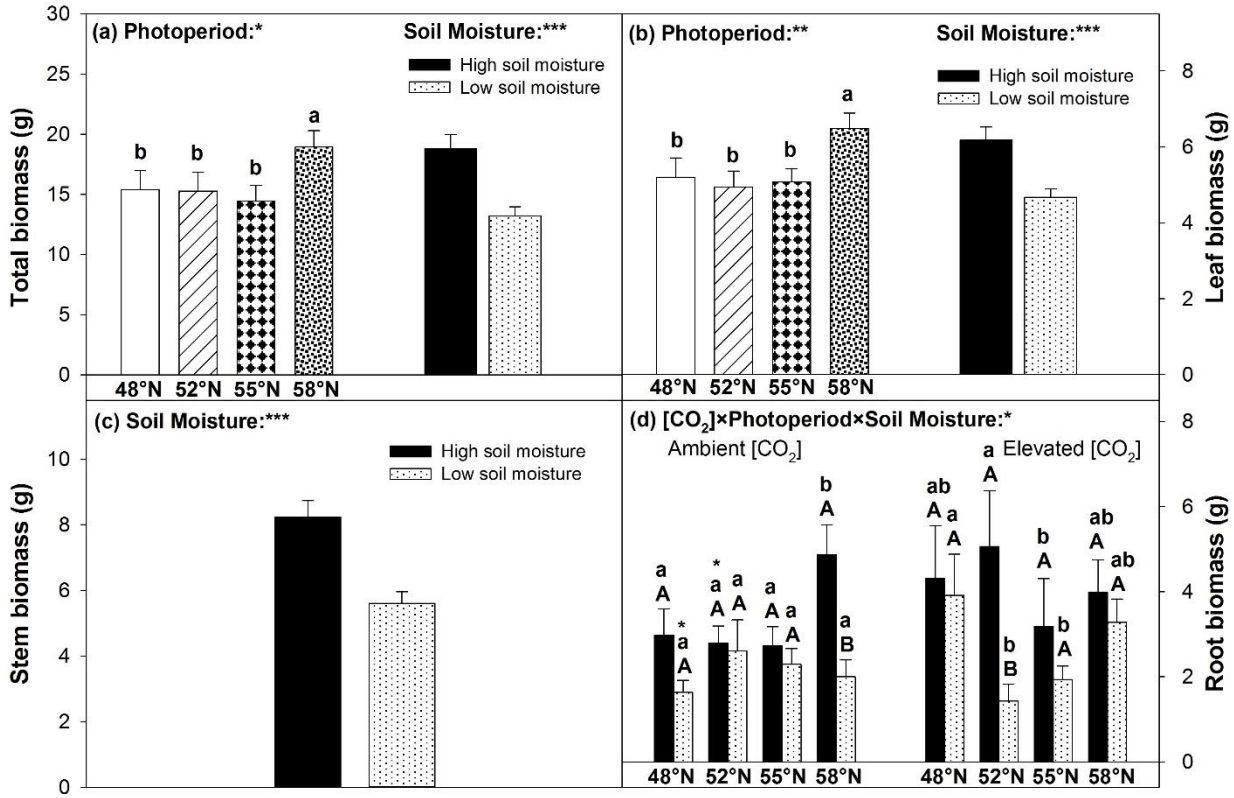

Figure 2.3 Total biomass (a) and leaf biomass (b) by four photoperiods (at 48 (seed origin), 52, 55, and 58°N) and two soil moisture (high vs. low), stem biomass (c) by two soil moisture regimes, and root biomass (d) by combinations of two [CO_2] (400 vs.1000 µmol mol-1), four photoperiods and two soil moisture regimes in trembling aspen seedlings (Mean + SE). Data were pooled for effects with $p > 0.05$: the data for Figures (a) and (b) were pooled for the 2 levels of [CO_2] and 2 levels of soil moisture treatments (n = 3 x 2 x 2 x 2 = 24). The data for Figure (c) were pooled for the 2 levels of [CO_2] and 4 levels of photoperiod treatments (n = 3 x 2 x 2 x 4 = 48). n for Figure (d) was 6 (3 x 2). Different letters above the bars in (a) and (b) indicate significant differences as shown by Fisher's Least Significant Difference (LSD) post hoc test (p <0.05). Different lowercase letters in (d) indicate significant differences among photoperiod within the same [CO_2] and soil moisture treatments (LSD test: p <0.05). Different uppercase letters in (d) indicate significant differences among soil moisture within the same [CO_2] and photoperiod treatments (LSD test: p <0.05). The asterisk above the bars in (d) indicate the significant differences among [CO_2] within the same photoperiod and soil moisture treatments (LSD test: p <0.05). The level of significance is shown (*p <0.05; ** p <0.01; *** p <0.001).

Longer photoperiods tended to reduce root: shoot ratio (RSR) and root mass ratio (RMR) under elevated [CO_2], although the trend seemed slightly different between low and high soil moisture (significant three-way interactions) (Table 1; Figures 4a, d). Low soil moisture generally

increased leaf mass ratio (LMR) (High: 0.34 ± 0.05, Low: 0.37±0.06) (Figure 4b), but tended to decrease RMR(High: 0.19 ±0.04, Low:0.17 ± 0.05), RSR (High: 0.23 ± 0.07, Low: 0.20 ±0.07), and stem mass ratio (SMR) (High:0.46 ± 0.04, Low: 0.44 ± 0.05) (Figure4a, c, d).

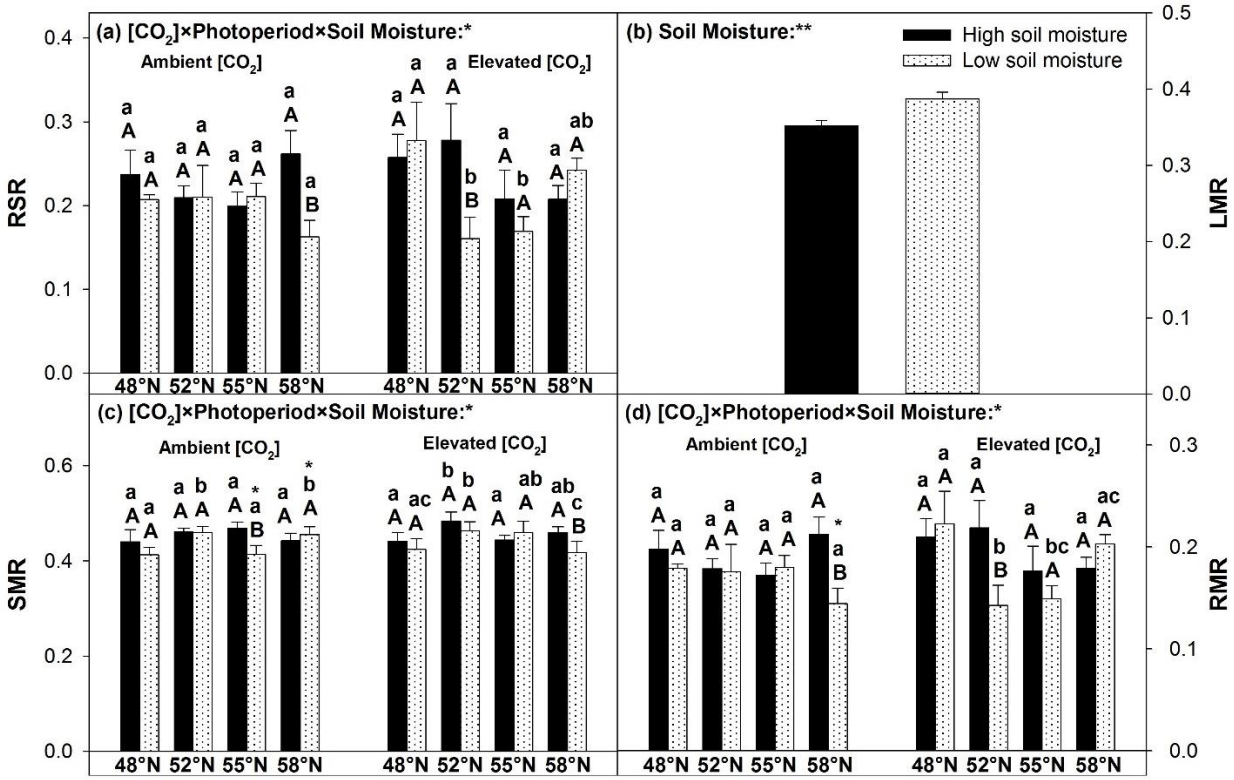

Figure 2.4 Root shoot ratio (RSR) (a), stem mass ratio (SMR) (c), and root mass ratio (RMR) (d) by combinations of two [CO_2] (400 vs. 1000 µmol mol-1), four photoperiods (at 48 (seed origin), 52, 55, and 58°N), and two soil moisture (high vs. low), and leaf mass ratio (LMR) (b) by two soil moisture in trembling aspen seedlings (Mean + SE). n = 6 for Figures a, c and d (3 seedlings per treatment combination x 2 replicates). n= 48 for Figure (b) because the data were pooled for the 2 levels of [CO_2] and 4 levels of photoperiod treatments because p >0.05 (n = 3 seedlings per treatment combination x 2 replications per combination x 2 [CO_2] x 4 photoperiods = 48). Different lowercase letters in (a), (c), and (d) indicate significant differences among photoperiod within the same [CO_2] and soil moisture treatments (LSD test: $p < 0.05$). Different uppercase letters in (a), (c), and (d) indicate significant differences among soil moisture within the same [CO_2] and photoperiod treatments (LSD test: $p < 0.05$). The asterisk above the bars in (c) and (d) indicate the significant differences among [CO_2] within the same photoperiod and soil moisture treatments (LSD test: $p < 0.05$). The level of significance is shown (*p <0.05; ** p <0.01; *** p <0.001).

Xylem cavitation resistance

The effects of [CO_2], photoperiod, and soil moisture on xylem cavitation resistance varied with the level of conductivity loss (Table 2). Under the EC, the seedlings in long photoperiods reached 20%, 50%, and 80% conductivity loss ($P_{20,}$ P_{50}, and P_{80}) at less negative water potentials (less cavitation resistant) than those at the seed origin (Figures 5, 6). Particularly, there was no overlap of CIs at P_{20}, P_{50} and P_{80} between 48˚N (P_{20} :-1.51MPa (1.11-1.83), P_{50} :-2.22MPa (CIs:1.75-2.32), P_{80} :-2.75MPa (2.43-3.20)) and 58˚N (P_{20} :-0.86MPa (0.62-1.12), P_{50} :-1.33MPa (CIs:1.09-1.53), P_{80} :-1.89MPa (1.62-2.22)) under EC, which indicates significant difference between the shortest and longest photoperiod treatments (Table 3; Figure 5e and h). Under the AC, however, the threshold water potentials of conductivity loss appeared to be more negative (more resistant to cavitation) at the photoperiod of the highest latitude but the differences were generally not significant (Table 3; Figure 6a-d). The seedlings in low soil moisture reached P_{80} at less negative water potential, however the CIs overlapped between high and low soil moisture (High: -2.3MPa (2.03-2.69), Low; -2.08MPa (1.80-2.42)) (Figure 3). Seedlings in low soil moisture had significantly lower maximum specific hydraulic conductivity (K_{smax}) (0.0020 ± 0.0013 mg mm^{-1} s^{-1} MPa^{-1}) than that of high soil moisture treatment (0.0014 ± 0.0013 mg mm^{-1} s^{-1} MPa^{-1}) (Figure 6).

Table 2.2 Summary of ANOVA for water potentials associated with 20%, 50% and 80% loss of conductivity (P_{20}, P_{50} and P_{80}, respectively), and maximum specific hydraulic conductivity (K_{smax}). The seedlings were exposed to two levels of [CO_2] (400 vs. 1000 µmol mol^{-1}), four photoperiod regimes (at 48.38°N (seed origin), 51.68°N, 54.98°N, and 58.28°N), and two soil moisture regimes (high (60%-75%) vs. low (13% -20%)). The numbers in bold are significant *p*-values at <0.05.

Variable		C (DF=1)	P (DF=3)	SM (DF=1)	C × P (DF=3)	C × SM (DF=1)	P × SM (DF=3)	C × P × SM (DF=3)
P_{20}	*F*	433	1.503	0.286	5.929	1.607	1.073	0.669
	P	**0.002**	0.307	0.607	**0.032**	0.241	0.413	0.594
P_{50}	*F*	17.360	1.022	1.648	7.488	2.387	0.891	0.697
	P	0.053	0.447	0.235	**0.019**	0.161	0.486	0.580
P_{80}	*F*	2.628	1.048	9.836	7.201	5.078	1.234	1.155
	P	0.246	0.437	**0.014**	**0.021**	0.054	0.359	0.385
K_{smax}	*F*	0.248	0.789	5.441	2.116	0.076	0.115	0.365
	P	0.668	0.543	**0.048**	0.200	0.790	0.949	0.780

*DF=degree of freedom

Table 2.3 Water potentials (average MPa) plus 95% confidence intervals at 20% (P_{20}), 50% (P_{50}), and 80% (P_{80}) loss in hydraulic conductivity of trembling aspen seedlings. Curves were fit for each individual seedling and the 95% confidence interval for P20, 50 and 80 were calculated.

Treatment		P20 (MPa) (95% CI)	P50 (MPa) (95% CI)	P80 (MPa) (95% CI)
Soil moisture	High	1.13 (0.82-1.40)	1.37 (1.39-1.87)	2.30 (2.03-2.69)
	Low	1.04 (0.74-1.31)	1.52 (1.24-1.72)	2.08 (1.80-2.42)
$[CO_2]$ × Photoperiod	Ambient × 48˚N	0.78 (0.55-1.07)	1.27 (1.03-1.50)	1.92 (1.65-2.30)
	Ambient × 52˚N	1.08 (0.84-1.24)	1.44 (1.25-1.63)	1.87 (1.70-2.20)
	Ambient × 55˚N	0.88 (0.61-1.17)	1.38 (1.12-1.56)	1.97 (1.72-2.26)
	Ambient × 58˚N	1.17 (0.81-1.58)	1.81 (1.45-2.03)	2.55 (2.17-3.02)
	Elevated × 48˚N	1.51 (1.11-1.83)	2.21 (1.75-2.32)	2.75 (2.43-3.20)
	Elevated × 52˚N	1.30 (0.96-1.51)	1.83 (1.55-2.00)	2.42 (2.13-2.77)
	Elevated × 55˚N	1.07 (0.75-1.36)	1.57 (1.28-1.79)	2.15 (1.86-2.48)
	Elevated × 58˚N	0.86 (0.62-1.12)	1.33 (1.09-1.53)	1.89 (1.62-2.22)

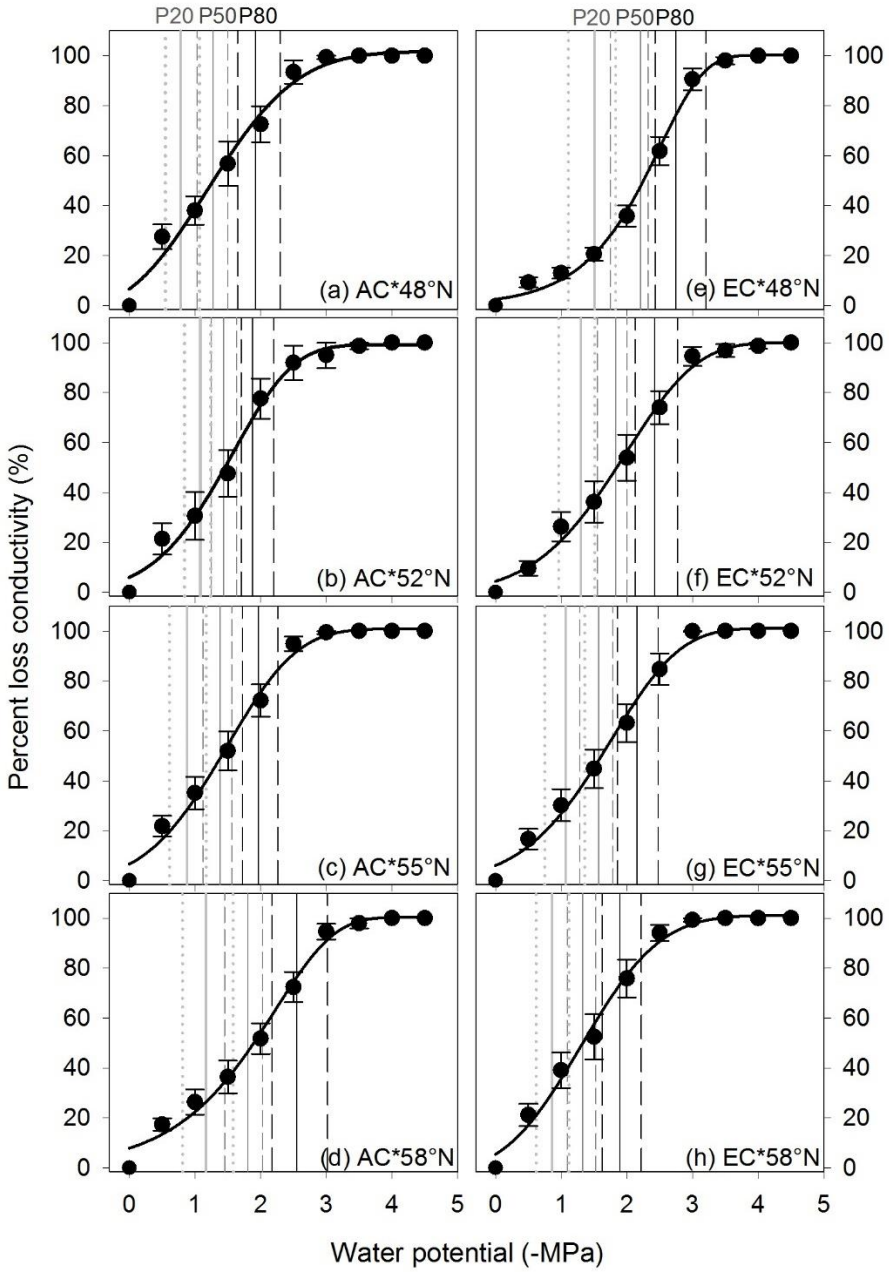

Figure 2.5 Hydraulic vulnerability curves showing the response of percent loss of stem hydraulic conductivity (%) to decreasing water potential (-MPa) in trembling aspen seedlings under the interactive effect of [CO_2] (ambient (AC) vs. elevated (EC)) and photoperiod (corresponding to 48, 52, 55 and 58 ˚N latitude). Each data point shows the mean PLC+SE at different water potential. Each panel was presented as n = 12: 3 seedlings per treatment combination and 2 replications per combination; the data were pooled for the 2 level of soil moisture treatment because the 3-way interaction was not statistically significant, 3 x 2 x 2 = 12).Vertical dotted/dashed lines indicate the 95% confidence interval for P20 (gray with dotted line), P50 (dark-gray with short-dash), and P80 (black with medium-dash) (estimated from the bootstrap).

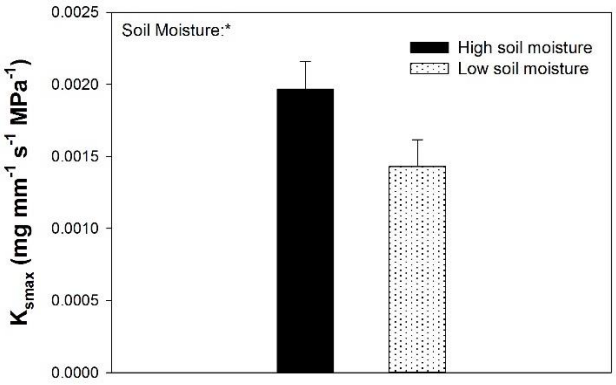

Figure 2.6 Maximum specific hydraulic conductivity (K_{smax}) of trembling aspen seedlings by two soil moisture (high and low). The data are presented as Mean + SE (n = 48: 3 seedlings per treatment combination and 2 replications per combination; the data were pooled for the 2 levels of [CO_2] and 4 levels of photoperiod treatments because the 3-way interaction was not statistically significant, 3 x 2 x 2 x 4 = 48).The level of significance is shown (*p <0.05).

2.5 Discussion

The first hypothesis was fully supported by my findings that the seedlings under the longest photoperiod (corresponding to 58˚N latitude) had the highest growth in height and biomasses among all the photoperiod regimes. The results are in general agreement with the findings of Oleksyn et al. (1992) on Scots pine (*Pinus sylvestris* L.). Specific leaf area (SLA), however, was the lowest for seedlings grown under the longest photoperiod. While growth rates are generally positively related to SLA, the opposite is occasionally reported (Wright et al. 1994, Thumma et al. 2001). The negative relationship between SLA and growth can be explained as that thicker leaves associated with low SLA may have more mesophyll cells per unit leaf area which will increase photosynthesis and therefore biomass production (Thumma et al. 2001, Anyia and Herzog 2004). A low SLA should reduce transpirational water loss, increase water use efficiency, and ameliorate the risk of desiccation, particularly for seedlings with high leaf biomass, which is the case in this study.

My results suggest that trembling aspen can physiologically acclimate to drought. The seedlings in the low soil moisture treatment had lower hydraulic conductivity. Low soil moisture reduces vessel diameter (Awad et al. 2010), resulting in lower water transport capacity or hydraulic efficiency (Tyree and Zimmermann 2002). While a high hydraulic conductivity generally promotes higher growth rates (Hacke and Sperry 2001, Tyree 2003) as demonstrated by the growth and biomass data in this study, the smaller vessel diameter associated with a low hydraulic conductivity can increase xylem resistance to cavitation, particularly freeze-thaw induced cavitation (Davis et al. 1999, Venturas et al. 2017). Fast-growing and short-lived pioneer species, such as trembling aspen, generally have high phenotypic xylem plasticity to acclimate to different soil moisture conditions (Plavcová and Hacke 2012; Schreiber et al. 2015). My results are generally consistent with the findings of other studies (Tyree and Zimmermann 2002; Nobel 2009; Hacke et al. 2016).

The results do not support the hypothesis that drought would increase xylem resistance to drought-induced cavitation. Although the specific hydraulic conductivity of the stem was lower in the low soil moisture treatment, there were no significant differences between the two soil moisture treatments in the water potentials for 20%, 50 and 80 % loss of hydraulic conductivity. This result is contrary to the expectation that high hydraulic conductivity is associated with large xylem vessels and low cavitation resistance (Wheeler et al. 2005, Markesteijn et al. 2011, Hacke et al. 2016, Pratt and Jacobsen 2017). However, cavitation resistance is more closely related to the characteristics of the pit membrane (Wheeler et al. 2005). It is possible that the result may vary with seedling age (Sperry et al. 1991). Since the rare largest membrane pore and the average size of pits in a vessel affect the tree's cavitation resistance (Wheeler et al. 2005), the difference

in cavitation resistance in different soil moisture treatments would be more apparent in larger trees.

My hypothesis on the effects of elevated [CO_2] and longer photoperiod on cavitation resistance was only partially supported: cavitation resistance decreased with longer photoperiods under the elevated [CO_2]. The seedlings grown under 58˚N reached a 50% loss of conductivity at high water potential (-1.33 MPa) under elevated [CO_2] comparing to 48˚N (-2.21 MPa). While the reasons for this result cannot be entirely explained, there are studies of the effect of [CO_2] and photoperiod on xylem anatomical structure, which may affect tree cavitation resistance (Hacke and Sperry 2001, Tyree and Zimmermann 2002, Cai and Tyree 2010). CO_2 elevations have been reported to increase xylem cell size in several *Populous spp*. (Luo et al. 2005, Kostiainen et al. 2014), which is not linked to changes in cavitation resistance. In *P. tremula,* CO_2 elevation did not affect the cavitation resistance (Tognetti et al. 1999). However, CO_2 elevations and longer photoperiods can increase the diameter of xylem conduits (Larson 1962, Handa et al. 2006, Kostiainen et al. 2014) but the effects of elevated [CO_2] vary greatly with changes in other environmental factors (Saxe et al. 1998, Norby et al. 1999, 2010, Curtis et al. 2000, Oren et al. 2001). In the present study, I did not characterize xylem anatomical changes in response to my experimental treatments, but I feel that an investigation on the response of xylem anatomy to the interactions of CO_2 elevation and longer photoperiods is warranted to further elucidate the physiological mechanisms of trembling aspen in the context of northward migration. Nonetheless, my results indicate that the xylem resistance to cavitation in trembling aspen could decline when the species migrate northward to higher latitudes under future climate conditions, which could impact the success of its migration or seed transfer as an adaptive strategy to climate change.

In summary, my results suggest that trembling aspen would have greater growth and biomass under a longer photoperiod when migrating to the north, but the growth enhancement may be offset by low soil moisture. However, the xylem resistance to cavitation decreased with increasing photoperiod regimes associated with higher latitudes under elevated [CO_2]. This, along with reduced hydraulic conductivity under low soil moisture, suggests that aspen could have lower resistance to water stress when migrating northward if drought becomes more frequent and severe in the northern regions (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). My results indicate that although temperature and precipitation patterns are often considered as main factors influencing future tree distribution, other factors that are part of species' niche such as photoperiod, [CO_2] and moisture variability likely influence the shift of species distribution. As my results were obtained from one-year-old seedlings under controlled greenhouse conditions, the results may not be directly applicable to large trees grown in the field. However, the value I found in the present study (e.g., -1.27MPa at P_{50} in AC × 48˚N) is similar to the values reported for other *Populus* species from both field and controlled environment studies (Cai and Tyree 2010, Hacke et al. 2010, Hillabrand et al. 2016). The consistency in results from different studies, particularly between the present study and field research by other researchers, suggests that the use of manipulative experiments to investigate the interactive effects of multiple factors on xylem vulnerability can be a powerful tool for examining how trees may respond to the novel environmental conditions of the future and/or at new locations. My results indicate the need to consider the interactive effects of [CO_2], photoperiod, and soil moisture for planning assisted migration and for projecting the future distribution of boreal forests.

2.6 Reference

Abeli, T., Orsenigo, S., Guzzon, F., Faè, M., Balestrazzi, A., Carlsson-Granér, U., Müller, J.V., and Mondoni, A. 2015. Geographical pattern in the response of the arctic-alpine *Silene suecica* (Cariophyllaceae) to the interaction between water availability and photoperiod. Ecol. Res. **30**(2): 327–335. doi:10.1007/s11284-014-1225-3.

Adams, H.D., Zeppel, M.J.B., Anderegg, W.R.L., Hartmann, H., Landhäusser, S.M., Tissue, D.T., et al. 2017. A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nature Eco. Evol. **1**(9): 1285-1291. doi:10.1038/s41559-017-0248-x.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., et al. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manage. **259**(4): 660–684. doi:10.1016/j.foreco.2009.09.001.

Anyia, A.O., and Herzog, H. 2004. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur. J. Agron. **20**(4): 327–339. doi:10.1016/S1161-0301(03)00038-8.

Arend, M., and Fromm, J. 2007. Seasonal change in the drought response of wood cell development in poplar. Tree Physiol. **27**(7): 985–992. doi:10.1093/treephys/27.7.985.

Awad, H., Barigah, T., Badel, E., Cochard, H., and Herbette, S. 2010. Poplar vulnerability to xylem cavitation acclimates to drier soil conditions. Physiologia Plantarum, **139**(3): 280–288. doi:10.1111/j.1399-3054.2010.01367.x.

Cai, J., and Tyree, M.T. 2010. The impact of vessel size on vulnerability curves: data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx. Plant Cell Environ. **33**(7): 1059–1069. doi:10.1111/j.1365-3040.2010.02127.x.

Centritto, M., Lucas, M.E., and Jarvis, P.G. 2002. Gas exchange, biomass, whole-plant water-use efficiency and water uptake of peach (*Prunus persica*) seedlings in response to elevated carbon dioxide concentration and water availability. Tree Physiol. **22**(10): 699–706. doi:10.1093/treephys/22.10.699.

Curtis, P.S., Vogel, C.S., Wang, X., Pregitzer, K.S., Zak, D.R., Lussenhop, J., et al. 2000. Gas Exchange, Leaf Nitrogen, and Growth Efficiency of *Populus tremuloides* in a Co2-Enriched Atmosphere. Ecol. Appl. **10**(1): 3–17. doi:10.1890/1051-0761(2000)010[0003:GELNAG]2.0.CO;2.

Davis, S.D., Sperry, J.S., and Hacke, U.G. 1999. The relationship between xylem conduit diameter and cavitation caused by freezing. Am. J. Bot. **86**(10): 1367–1372.

Ennajeh, M., Simões, F., Khemira, H., and Cochard, H. 2011. How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms? Physiologia Plantarum, **142**(3): 205–210. doi:10.1111/j.1399-3054.2011.01470.x.

Fichot, R., Barigah, T.S., Chamaillard, S., Thiec, D.L., Laurans, F., Cochard, H., and Brignolas, F. 2010. Common trade-offs between xylem resistance to cavitation and other physiological traits do not hold among unrelated *Populus deltoides* ×*Populus nigra* hybrids. Plant Cell Environ. **33**(9): 1553–1568. doi:10.1111/j.1365-3040.2010.02164.x.

Gestel, N.C.V., Nesbit, A.D., Gordon, E.P., Green, C., Paré, P.W., Thompson, L., Peffley, E.B., and Tissue, D.T. 2005. Continuous light may induce photosynthetic downregulation in onion – consequences for growth and biomass partitioning. Physiologia Plantarum, **125**(2): 235–246. doi:10.1111/j.1399-3054.2005.00560.x.

Hacke, U.G., Plavcová, L., Almeida-Rodriguez, A., King-Jones, S., Zhou, W., and Cooke, J.E.K. 2010. Influence of nitrogen fertilization on xylem traits and aquaporin expression in stems of hybrid poplar. Tree Physiol. **30**(8): 1016–1025. doi:10.1093/treephys/tpq058.

Hacke, U.G., and Sperry, J.S. 2001. Functional and ecological xylem anatomy. Perspect. Plant Ecol. Evol. Syst. **4**(2): 97–115. doi:10.1078/1433-8319-00017.

Hacke, U.G., Spicer, R., Schreiber, S.G., and Plavcová, L. 2016. An ecophysiological and developmental perspective on variation in vessel diameter. Plant Cell Environ.: 40:831-845. doi:10.1111/pce.12777.

Handa, I.T., Körner, C., and Hättenschwiler, S. 2006. Conifer stem growth at the altitudinal treeline in response to four years of CO_2 enrichment. Global Change Biol. **12**(12): 2417–2430. doi:10.1111/j.1365-2486.2006.01258.x.

Hillabrand, R.M., Hacke, U.G., and Lieffers, V.J. 2016. Drought-induced xylem pit membrane damage in aspen and balsam poplar. Plant Cell Environ. **39**(10): 2210–2220. doi:10.1111/pce.12782.

Hogg, E.H., Brandt, J.P., and Michaelian, M. 2008. Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Can. J. For. Res. **38**(6): 1373–1384. doi:10.1139/X08-001.

Klein, T., Cohen, S., and Yakir, D. 2011. Hydraulic adjustments underlying drought resistance of *Pinus halepensis*. Tree Physiol. **31**(6): 637–648. doi:10.1093/treephys/tpr047.

Kostiainen, K., Saranpää, P., Lundqvist, S.-O., Kubiske, M.E., and Vapaavuori, E. 2014. Wood properties of *Populus* and *Betula* in long-term exposure to elevated CO_2 and O_3. Plant Cell Environ. **37**(6): 1452–1463. doi:10.1111/pce.12261.

Kozlowski, T.T. 1968. Water deficits and plant growth. New York : Academic Press.

Kozlowski, T.T., and Pallardy, S.G. 2002. Acclimation and adaptive responses of woody plants to environmental stresses. Bot. Rev. **68**(2): 270–334. doi:10.1663/0006-8101(2002)068[0270:AAAROW]2.0.CO;2.

Larson, P.R. 1962. The indirect effect of photoperiod on tracheid diameter in *Pinus resinosa*. Am. J. Bot. **49**(2): 132–137. doi:10.1002/j.1537-2197.1962.tb14918.x.

Luo, Z.-B., Langenfeld-Heyser, R., Calfapietra, C., and Polle, A. 2005. Influence of free air CO_2 enrichment (EUROFACE) and nitrogen fertilisation on the anatomy of juvenile wood of three poplar species after coppicing. Trees, **19**(2): 109–118. doi:10.1007/s00468-004-0369-0.

Markesteijn, L., Poorter, L., Paz, H., Sack, L., and Bongers, F. 2011. Ecological differentiation in xylem cavitation resistance is associated with stem and leaf structural traits. Plant Cell Environ. **34**(1): 137–148. doi:10.1111/j.1365-3040.2010.02231.x.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., et al. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive

while others succumb to drought? New Phytologist, **178**(4): 719–739. doi:10.1111/j.1469-8137.2008.02436.x.

McKenney, D.W., Pedlar, J.H., Lawrence, K., Campbell, K., and Hutchinson, M.F. 2007. Potential impacts of climate change on the distribution of North American trees. BioScience, **57**(11): 939–948. doi:10.1641/B571106.

McKenney, D.W., Pedlar, J.H., Rood, R.B., and Price, D. 2011. Revisiting projected shifts in the climate envelopes of North American trees using updated general circulation models. Global Change Biol. **17**(8): 2720–2730. doi:10.1111/j.1365-2486.2011.02413.x.

Michaelian, M., Hogg, E.H., Hall, R.J., and Arsenault, E. 2011. Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. Global Change Biol. **17**(6): 2084–2094. doi:10.1111/j.1365-2486.2010.02357.x.

Newaz, M., Dang, Q.-L., and Man, R. 2017. Jack pine becomes more vulnerable to cavitation with increasing latitudes under doubled CO_2 concentration. Botany, **96**(2): 111–119. doi:10.1139/cjb-2017-0142.

Nobel P.S. (2009) Physicochemical and Environmental Plant Physiology, 4th edn. Academic Press/Elsevier, San Diego, CA, USA

Norby, R.J., Warren, J.M., Iversen, C.M., Medlyn, B.E., and McMurtrie, R.E. 2010. CO2 enhancement of forest productivity constrained by limited nitrogen availability. Proc. Natl. Acad. Sci. U.S.A. **107**(45): 19368–19373. doi:10.1073/pnas.1006463107.

Norby, R.J., Wullschleger, S.D., Gunderson, C.A., Johnson, D.W., and Ceulemans, R. 1999. Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ. **22**(6): 683–714. doi:10.1046/j.1365-3040.1999.00391.x.

Oleksyn, J., Tjoelker, M.G., and Reich, P.B. 1992. Growth and biomass partitioning of populations of European *Pinus sylvestris* L. under simulated 50˚ and 60˚ N daylengths: evidence for photoperiodic ecotypes. New Phytologist, **120**(4): 561–574. doi:10.1111/j.1469-8137.1992.tb01806.x.

Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., et al. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature, **411**(6836): 469–472. doi:10.1038/35078064.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., et al. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited By*R.K. Pachauri and L. Meyer. IPCC, Geneva, Switzerland.

Perala, D.A. 1990. Populus tremuloides Michx.: quaking aspen. In silvics of North America. Vol. 2. Technical Coordinators: R.M.Burns and B.H. Honkala. U.S. Dep. Agric. Agric. Handb. 654. pp. 555–569.

Peterson, E.B., and Peterson, N.M. 1992. Ecology, management and use of aspen and balsam poplar in the prairie provinces, Canada. Special Report 1, Forestry Canada, Northwest Region, Northern Forestry Centre, Edmonton, Alberta Vol. 1, 252 p.

Plavcová, L., and Hacke, U.G. 2012. Phenotypic and developmental plasticity of xylem in hybrid poplar saplings subjected to experimental drought, nitrogen fertilization, and shading. J. Exp. Bot. **63**(18): 6481–6491. doi:10.1093/jxb/ers303.

Pratt, R.B., and Jacobsen, A.L. 2017. Conflicting demands on angiosperm xylem: Tradeoffs among storage, transport and biomechanics. Plant Cell Environ. **40**(6): 897–913. doi:10.1111/pce.12862.

Rigling, A., Eilmann, B., Koechli, R., and Dobbertin, M. 2010. Mistletoe-induced crown degradation in Scots pine in a xeric environment. Tree Physiol. **30**(7): 845–852. doi:10.1093/treephys/tpq038.

Saxe, H., Ellsworth, D.S., and Heath, J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. The New Phytologist, **139**(3): 395–436.

Schreiber, S.G., Hacke, U.G., and Hamann, A. 2015. Variation of xylem vessel diameters across a climate gradient: insight from a reciprocal transplant experiment with a widespread boreal tree. Funct. Ecol. **29**(11): 1392–1401. doi:10.1111/1365-2435.12455.

Sperry, J.S., Donnelly, J.R., and Tyree, M.T. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ. **11**(1): 35–40. doi:10.1111/j.1365-3040.1988.tb01774.x.

Sperry, J.S., Nichols, K.L., Sullivan, J.E.M., and Eastlack, S.E. 1994. Xylem embolism in ring-porous, diffuse-porous, and coniferous trees of Northern Utah and interior Alaska. Ecology, **75**(6): 1736–1752. doi:10.2307/1939633.

Sperry, J.S., Perry, A.H., and Sullivan, J.E.M. 1991. Pit membrane degradation and air-embolism formation in ageing xylem vessels of *Populus tremuloides* Michx. J. Exp. Bot. **42**(11): 1399–1406. doi:10.1093/jxb/42.11.1399.

Sperry, J.S., and Saliendra, N.Z. 1994. Intra- and inter-plant variation in xylem cavitation in *Betula occidentalis*. Plant Cell Environ. **17**(11): 1233–1241. doi:10.1111/j.1365-3040.1994.tb02021.x.

Sperry, J.S., and Sullivan, J.E.M. 1992. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous, and conifer species. Plant Physiol. **100**(2): 605–613. doi:10.1104/pp.100.2.605.

Thumma, B.R., Naidu, B.P., Chandra, A., Cameron, D.F., Bahnisch, L.M., and Liu, C. 2001. Identification of causal relationships among traits related to drought resistance in *Stylosanthes scabra* using QTL analysis. J. Exp. Bot. **52**(355): 203–214. doi:10.1093/jexbot/52.355.203.

Tognetti, R., Longobucco, A., and Raschi, A. 1999. Seasonal embolism and xylem vulnerability in deciduous and evergreen Mediterranean trees influenced by proximity to a carbon dioxide spring. Tree Physiol. **19**(4–5): 271–277. doi:10.1093/treephys/19.4-5.271.

Tyree, M.T. 2003. Hydraulic limits on tree performance: transpiration, carbon gain and growth of trees. Trees, **17**(2): 95–100. doi:10.1007/s00468-002-0227-x.

Tyree, M.T., and Zimmermann, M.H. 2002. Xylem structure and the ascent of sap. 2nd edition. Springer-Verlag, Berlin Heidelberg.

Venturas, M.D., Sperry, J.S., and Hacke, U.G. 2017. Plant xylem hydraulics: What we understand, current research, and future challenges. Journal of Integrative Plant Biology, **59**(6): 356–389. doi:10.1111/jipb.12534.

Vilagrosa, A., Chirino, E., Peguero-Pina, J.J., Barigah, T.S., Cochard, H., and Gil-Pelegrín, E. 2012. Xylem cavitation and embolism in plants living in water-limited ecosystems. *In* Plant responses to drought stress. *Edited by* R. Aroca. Springer Berlin Heidelberg. pp. 63–109. doi:10.1007/978-3-642-32653-0_3.

Wheeler, J.K., Huggett, B.A., Tofte, A.N., Rockwell, F.E., and Holbrook, N.M. 2013. Cutting xylem under tension or supersaturated with gas can generate PLC and the appearance of rapid recovery from embolism. Plant Cell Environ. **36**(11): 1938–1949. doi:10.1111/pce.12139.

Wheeler, J.K., Sperry, J.S., Hacke, U.G., and Hoang, N. 2005. Inter-vessel pitting and cavitation in woody *Rosaceae* and other vesselled plants: a basis for a safety versus efficiency trade-off in xylem transport. Plant Cell Environ. **28**(6): 800–812. doi:10.1111/j.1365-3040.2005.01330.x.

Wright, G.C., Rao, R.C.N., and Farquhar, G.D. 1994. Water-use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop Sci. **34**(1): 92–97. doi:10.2135/cropsci1994.0011183X003400010016x.

3 Chapter 3: Photoperiod and elevated [CO_2] influence physiological responses to drought in trembling aspen: implications to climate change-induced migration

3.1 Abstract

The success of climate change-induced tree migration is influenced by genetic and environmental factors which often interact with each other. However, the interactive effects of these factors are currently not well understood. In this study, I investigated the morphological and physiological responses of *Populus tremuloides* Michx. to interactions of atmospheric CO_2 concentration ([CO_2]), photoperiod, and soil moisture regimes from the perspective of climate change-induced northward migration. Seedlings were exposed to two [CO_2], (AC: 400 vs. EC: 1000 μmol mol^{-1}), four photoperiod regimes corresponding to 48 (seed origin), 52, 55, and 58°N latitude and two soil moisture regimes (HSM: high vs. LSM: low) for two growing seasons in environment-controlled greenhouses. I have found that photoperiod and [CO_2] significantly affected responses to water stress and *vice versa*. Stomatal conductance (g_s) and its reduction in response to LSM declined with increasing photoperiod. Surprisingly, LSM resulted in an upregulation of photosynthesis as indicated by increases in the maximum rate of Rubisco carboxylation and maximum rate of electron transport for RuBP regeneration, and the magnitude of the upregulation increased with increasing photoperiod. LSM induced photosynthetic upregulation was accompanied by an increase in biomass allocation to roots and a reduction in g_s. LSM increased the instantaneous water-use efficiency (iWUE) and the increase was greater under EC. While LSM increased photosynthesis in AC and EC in July, the increase was significant only under EC in August. EC increased iWUE in both soil moisture treatments in July, but the increase was significant only under LSM in August. Furthermore, EC enhanced P_n only under LSM but not HSM. The counterintuitive physiological responses to drought indicate

the critical and predominant role of morphological acclimation in trembling aspen in response to LSM.

Additional keywords: tree migration; gas exchange; [CO_2]; soil moisture; *Populus tremuloides* Michx.; photoperiod; morphology.

3.2 Introduction

The increase in atmospheric CO_2 concentration ([CO_2]) and change in water availability associated with increasing temperature and changes in precipitations are expected to substantially alter the performance of trees by affecting their physiology, phenology, and growth (Keeling et al. 1996, Myneni et al. 1997, Huang et al. 2007, Delbart et al. 2008). These changes have led to the northward shift of the climate envelopes for boreal trees (McKenney et al. 2007, 2011). However, northward migration will expose trees to substantial changes in factors that are associated with latitudes such as photoperiod, as well as changes in soil water supply and air dryness in many parts of North America (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). The new set of environmental conditions can hinder or promote the survival and growth of trees and thus influence the geographic distribution of tree species (Thomas and Vince-Prue 1996, Jach et al. 2001). However, the physiological and/or growth responses of boreal tree species to these potential changes are not well understood.

The migration of plants in response to climate change may require the acclimation of plant physiology, morphology, and/or biomass allocation to cope with the changes in environmental conditions (Lafleur et al. 2010). For instance, the predicted more frequent and prolonged water stress in many regions of North America in the future (Pachauri, Allen, Barros, Broome, Cramer, et al. 2014) may hamper the establishment of tree seedlings during migration if the trees are

unable to acclimate to the new condition. Higher [CO_2], on the other hand, may lower the impact of water stress via reducing stomatal aperture and drought limitation to photosynthesis (Robredo et al. 2007). In addition, plants exposed to elevated [CO_2] often allocate a greater proportion of biomass to roots to improve access to soil moisture (Stulen and Den Hertog 1993, Saxe et al. 1998). Since the photoperiod is longer in the growing season at higher latitudes, the impact of water stress may be even greater because of the extended period of transpirational water loss. However, it is unknown whether the impact of longer photoperiod under the effect of water stress can be compensated by the positive effect of [CO_2] elevation.

Photoperiod can regulate growth and phenology in photoperiod sensitive plant species (Nelson et al. 2010). Long photoperiods can stimulate plant growth (Oleksyn et al. 1992, Johnsen and Seiler 1996) and increase resource allocation to above-ground organs (Hay 1990, Thomas and Vince-Prue 1996). It has also been reported that photoperiods alter the rate of photosynthesis and leaf nitrogen content (Comstock and Ehleringer 1986). However, photoperiod is determined by latitude and does not change with climate change, while moisture availability and other environmental factors are affected by climate change (Pachauri, Allen, Barros, Broome, Cramer, et al. 2014). Plants have evolved various mechanisms to acclimate to moisture stress, from molecular, biochemical, physiological, to morphological mechanisms (Passioura 1996). In short- to medium-terms, plants respond or acclimate to drought physiologically, ranging from stomatal closure to metabolic adjustments by tight coordination between photosynthesis, respiration, and photorespiration (Passioura 1996). Plants or leaves that have developed during water stress may have a higher concentration of nitrogen and Rubisco enzyme in the leaf, leading to photosynthetic up-regulation on the leaf area basis (Panković et al. 1999, Kitao et al. 2003, Aranda et al. 2005, Kitaoka and Koike 2005). Plants exposed to moisture stress generally

increase a greater proportion of biomass to roots to better access soil water (Poorter et al. 2012, Hertel et al. 2013), whereas longer photoperiod regime increases the allocation of biomass to shoots (Hay 1990, Thomas and Vince-Prue 1996). Since the partitioning of biomass between above- and below-ground organs underlie a trade-off (Poorter and Nagel 2000, Craine 2009), an interesting question is how photoperiod and moisture stress will interactively affect biomass allocation, particularly under the higher [CO_2] of the future. Further, since higher [CO_2] generally increases photosynthetic carbon fixation in C_3 plants (Jach et al. 2001), elevated [CO_2] is expected to further increase biomass allocation to roots under water stress and could influence the interactive effect of photoperiod and moisture stress. A good understanding of the responses in the physiology, growth, biomass, and biomass allocation of tree seedlings to simultaneous changes in photoperiod, water, and CO_2 elevation is particularly important for understanding the potential performance of trees under the scenario of climate change-induced northward migration or seed transfer in the projected future conditions.

Trembling aspen (*Populus tremuloides* Michx.) is the most abundant deciduous tree species in Canada's boreal forest (Perala 1990, Hogg et al. 2002) and a commercially important source of the wood fiber. The species also has great cultural values for indigenous people and is an important species for wildlife and biodiversity. Declines and die-back of trembling aspen have been observed across large areas of west-central Canada due to recent severe drought spells, coupled with other stresses such as insect outbreaks and pathogens (Hogg et al. 2008, Marchetti et al. 2011, Worrall et al. 2013). The fact that trembling aspen is very sensitive to water availability and the boreal region is predicted to become drier in the future (Sherwood and Fu 2014, Seager et al. 2014, Wang et al. 2014) suggests that managing trembling aspen will be more challenging in the future. In this study, I investigated whether longer photoperiods would

exacerbate the negative effect of moisture stress on the physiology and growth of trembling aspen and whether [CO_2] elevation could ameliorate the negative effect of moisture stress and/or moderate the effect of longer photoperiod on biomass partitioning. I also attempted to determine what would be the primary mechanism or mechanisms responsible for the response or acclimation of the species to the simultaneous changes in these three factors.

3.3 Materials and Methods

3.3.1 Plant materials

I collected catkins of trembling aspen (*Populus tremuloides* Michx.) from 10 free-pollinated trees on June 9th, 2016 in Thunder Bay, Ontario, Canada (48.38°N, 89.25°W). The catkins were air-dried at room temperature for five days, and seeds were extracted using the method of Moench (1999). All seeds were mixed and stored in a sealed glass bottle at -4°C.

Seeds were sown in horticultural trays filled with a 1:1 (v/v) mixture of peat moss and vermiculite in a greenhouse at Lakehead University. The greenhouse was maintained at 24/14 (±2) °C (day/night) temperature, and the natural photoperiod was extended to 16h by using high-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada). All trays were well-watered. After 18 days from the completion of germination, the seedlings were individually transplanted to circular pots (12 cm in height, 12 cm and 9.5 cm top and bottom diameter, respectively) filled with a mixture of peat moss and vermiculite (7:3 v/v).

3.3.2 Experiment design

The experiment was conducted in four greenhouses at the Thunder Bay campus of Lakehead University. The treatments comprised two levels of [CO_2] (400 (ambient) vs. 1000 (elevated) μmol mol^{-1}), four photoperiod regimes corresponding to the photoperiod at 48.38 (seed origin)

(48°N), 51.68 (52°N), 54.98 (55°N), and 58.28°N (58°N), and two soil moisture regimes (high vs. low). The experiment design was a split-split plot design where the [CO_2] was the main plot, photoperiod was sub-plot, and soil moisture was sub-sub-plot. Each of the two [CO_2] treatments were randomly assigned to two of the four greenhouses (2 replicates for each [CO_2]). The photoperiod treatment was nested within the [CO_2] treatment, and soil moisture was nested within the photoperiod.

3.3.3 Treatments and the greenhouse environment controls

The environmental condition of each greenhouse was individually controlled using the Argus Titan Environment Control System (Argus Controls Systems Ltd., Surrey, BC, Canada). The elevated [CO_2] was achieved with the model GEN2E gas CO_2 generators (Custom Automated Products Inc., Riverside, California, USA). The photoperiod of each greenhouse was set to the longest photoperiod of the four treatments (58.28°N). The natural light level was supplemented (on cloudy days, early mornings, and late evenings) and natural day length was extended using high-pressure sodium lamps (Model LR48877, P.L. Systems, Grimspy, ON, Canada). The shorter photoperiods were attained by shading with neutral density shading cloth. For each photoperiod treatment, a wooden frame was installed to facilitate the shading. Efforts were made to ensure that no light would leak into the shaded space in the shorter photoperiod treatments. The photoperiod setting was adjusted weekly based on the calculated photoperiod for each of the four latitudes. The soil volumetric water content was monitored using a Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK). Soil volumetric water content was maintained between 60% and 75% of the field capacity for the high moisture treatment and between 13% and 20% of the field capacity for the low soil moisture treatment.

The experiment was carried out for two growing seasons, November 1st to April 16th, 2016/17 (emulating the natural environmental conditions of June 7th to November 20th) and November 1st to May 15th, 2017/18 (emulating the natural environmental conditions of April 26th to November 7th). The emulated natural dates were used in the subsequent presentations of the paper. Temperature adjustments were synchronized with the adjustments of photoperiod based on the weekly averages of hourly temperatures in the past ten years for each of the latitudes (Environment Canada records for Thunder Bay, ON). The daily temperature in each greenhouse was ramped at 6-hour set points. I fertilized seedlings in three phases, during which the fertilizer solution contained 33 mg N L^{-1}, 73 mg P L^{-1} and 55 mg K L^{-1} for the establishment phase (June 7 to June 21 in the first growing season and April 26 to May 25 in the second growing season); 100 mg N L^{-1}, 60 mg P L^{-1}, 150 mg K L^{-1}, 80 mg Ca L^{-1}, 40 mg Mg L^{-1} and 60 mg S L^{-1} for the fast-growing phase (June 22 to August 31 and May 26 to August 31 for the growing seasons), and of 33 mg N L^{-1}, 44 mg P L^{-1} and 83 mg K L^{-1} for the hardening phase (September 1 to 25) (Landis 1989, Jacobs and Landis 2009).

After being cold hardened at the end of the first growing season, seedlings were stored in walk-in cold storage at -4°C. The seedlings were covered with plastic sheets to avoid desiccation during the storage. The seedlings were transferred into larger pots (18 cm in height, 16 cm top- and 14 cm bottom-diameter) in the second growing season.

3.3.4 Measurements

Chlorophyll fluorescence and foliar gas exchange measurements

Foliar gas exchange was measured in July and again in August, and chlorophyll fluorescence was measured only in August of the second growing season. Three seedlings were randomly

selected from each treatment-replication combination for the measurements. A healthy and fully expanded leaf (8^{th} or 9^{th} from the top) was measured. Gas exchange was measured using a PP-Systems CIRAS-3 open gas exchange system with PLC3 Universal Leaf Cuvette (PP Systems, Amesbury, MA). The measurements were made at 22 °C air temperature, 50% RH, 800 μmol $m^{-2}s^{-1}$ PAR and sequentially at the following [CO_2]: 400, 300, 250, 200, 150, 100, 50, 400, 450, 550, 650, 750, 850, 1000, 1200, 1500, and 1800 μmol mol^{-1} CO_2. The measurements were carried between 8:00 AM and 3:00 PM when tests showed gas exchange measurements were relatively stable. The net photosynthetic rate at growth [CO_2] (P_n) and P_n at the ambient [CO_2] (P_{n400}) were extracted from the A/Ci data for each sample tree. Stomatal conductance (g_s), transpiration rate (E), instantaneous water use efficiency (iWUE), and intercellular/ambient [CO_2] ratio at the corresponding growth [CO_2] were used for subsequent analyses and presentations.

Maximum (Fm) and minimum (Fo) fluorescence yields of dark-adapted leaves were measured prior to the gas exchange measurement using a Hansatech FMS-2 portable pulse-modulated fluorometer (Hansatech Instruments Ltd. Norfolk, UK) on the same leaf on which the gas exchange was measured. The leaves were dark-adapted for at least 40 minutes for this measurement. The potential maximum quantum efficiency of photosystem II (PSII) was determined as (Fm – Fo)/Fm.

Growth and biomass allocation

I selected three seedlings from each treatment and replication combination at the end of the second growing season for height and root collar diameter (RCD) measurements. The sample trees were then harvested and separated into leaves, stem, and roots. Total leaf area and the

number of leaves per tree were determined using the Regent Instruments WinFolia system (Quebec, Canada). Roots were washed clean. The dry mass of leaf, stem, and root was determined after being oven-dried at 80°C for 48 hours. The following variables were determined: specific leaf area (SLA = total leaf area / total leaf dry mass), leaf area ratio (LAR = total leaf area /total seedling dry mass), leaf mass ratio (LMR= leaf mass/seedling dry mass), stem mass ratio (SMR= stem dry mass/ total seedling dry mass), root mass ratio (RMR= root dry mass/total seedling dry mass), and root to shoot ratio (RSR= root dry mass/ total leaf and stem dry mass).

A/Ci curve fitting

The A/Ci data was analyzed using the "fitaci" function of the "plantecophys" package (Duursma, 2015) on the R 3.5.0 software package (R Development Core Team, 2018). The "default" fitting method was used to estimate the maximum rate of Rubisco carboxylation (V_{cmax}), and the maximum rate of electron transport for RuBP regeneration under saturating light (J_{max}) for each seedling (Duursma, 2015).

3.3.5 Data Analysis

The data were examined graphically for the normality of distribution (probability plots of residuals) and homogeneity of variance (scatter plots) using the R software (Version 3.5.0, R Development Core Team 2018) before being subjected to the analysis of variance (ANOVA). The effects of [CO_2], photoperiod regimes, and soil moisture were all considered as having "fixed factors" in the ANOVA. An effect was considered significant if $p \leq 0.05$. When the ANOVA showed a significant ($p \leq 0.05$) photoperiod effect or a significant interaction, the Fisher's Least Significant Difference (LSD) post-hoc test was used to compare individual means.

All the analyses were performed using R software (Version 3.5.0, R Development Core Team 2018).

3.4 Results

Growth and biomass allocation

Seedling height generally increased with increases in photoperiod, although the difference was only significant between the shortest (seed origin) and the longest photoperiod (10° north of the seed origin) (Table 3.1, Fig. 3.1a). Low soil moisture (LSM) reduced height growth, root collar diameter (RCD), leaf size, and total leaf area by 32.5%, 32.5%, 49.6%, and 86.3%, respectively (Figs. 3.1a-d). LSM reduced leaf, stem, root, and total biomass by 95%, 101.3%, 63.6%, and 87.8%, respectively (Table 3.1, Fig. 3.2a). However, the reduction of root biomass by LSM was smaller under EC than AC (39% vs. 96.1%, Fig. 2a). In relative terms, LSM reduced biomass allocation to stems (SMR), but increased the allocation to roots (RMR), leading to a higher root-shoot ratio (RSR) (Table 3.2, Fig. 3.2b).

Table 3.1 ANOVA results (*p* values) for the effects of [CO_2] (C), photoperiod (P), soil moisture (SM) and their interactions on height, root collar diameter (RCD), leaf size, total leaf area (Total LA), specific leaf area (SLA), leaf biomass (Leaf DW), stem biomass (stem DW), root biomass (root DW), leaf mass ratio (LMR), shoot mass ratio (SMR), root mass ratio (RMR), and shoot root ratio (SRR). The seedlings were exposed to two levels of [CO_2] (400 vs. 1000 µmol mol^{-1}), four photoperiod regimes (at 48 (seed origin), 52, 55, and 58°N), and two soil moisture regimes (high vs. low). The numbers in bold are significant at <0.05.

Variable	C (*DF=1)	P (DF=3)	SM (DF=1)	C × P (DF=3)	C × SM (DF=1)	P × SM (DF=3)	C × P × SM (DF=3)
Height	0.680	**0.043**	**<0.001**	0.438	0.262	0.662	0.553
RCD	0.266	0.923	**<0.001**	0.959	0.631	0.940	0.545
Leaf size	0.711	0.191	**<0.001**	0.127	0.059	0.885	0.232
Total LA	0.463	0.159	**<0.001**	0.283	0.115	0.475	0.511
SLA	0.250	0.240	0.552	0.392	0.122	0.275	0.452
Leaf DW	0.295	0.186	**<0.001**	0.269	0.826	0.314	0.808
Stem DW	0.399	0.694	**<0.001**	0.408	0.239	0.793	0.102
Root DW	0.549	0.639	**<0.001**	0.173	**0.043**	0.999	0.204
Total DW	0.435	0.875	**<0.001**	0.275	0.189	0.805	0.168
LMR	0.888	0.184	0.368	0.352	0.073	0.078	0.062
SMR	0.847	0.149	**0.007**	0.892	0.751	0.493	0.180
RMR	0.998	0.118	**0.024**	0.096	0.376	0.773	0.815
RSR	0.966	0.150	**0.025**	0.107	0.391	0.776	0.827

*DF=degree of freedom

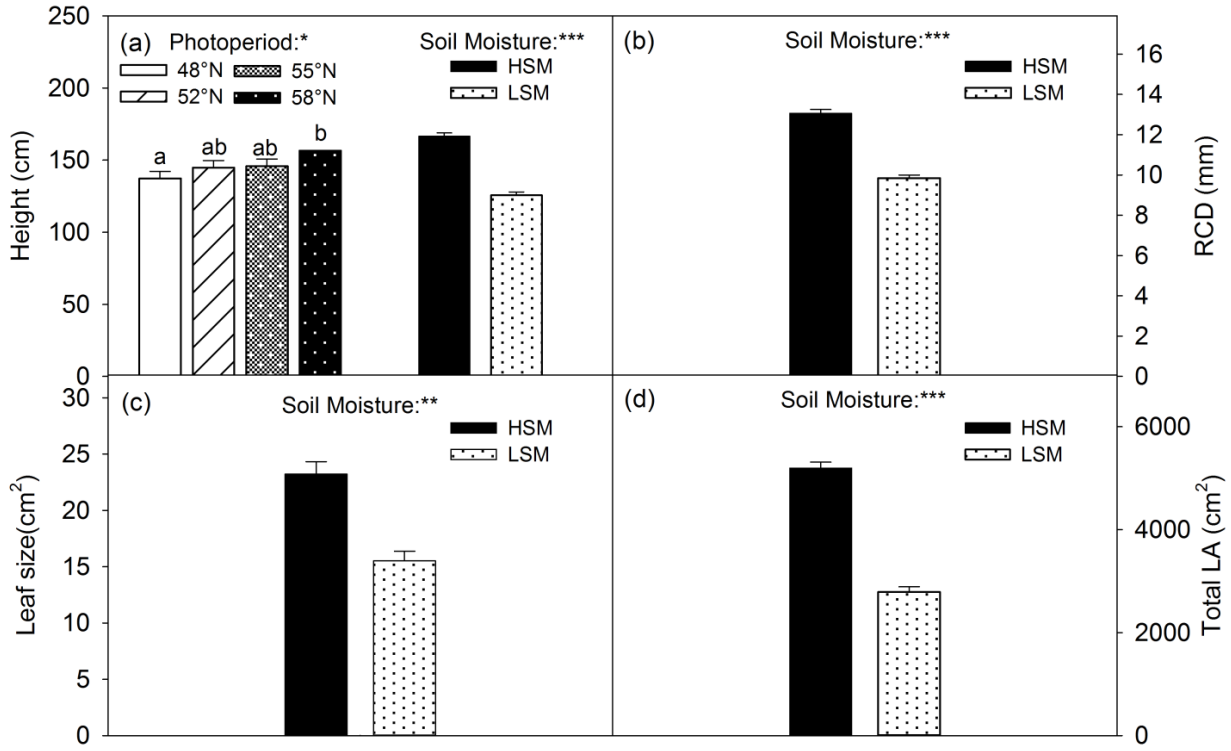

Figure 3.1 Mean (+SE) height growth (a), root collar diameter (RCD) (b), leaf size (c), total LA (d) of trembling aspen seedlings grown under two levels of [CO_2] (ambient vs. elevated), four photoperiod regimes (at 48(seed origin), 52, 55, and 58°N) and two levels of soil moisture (high vs. low) for two growing seasons. Data are pooled across [CO_2] and soil moisture for (a)-left (n =24: three seedlings with two replications per treatment, two levels of [CO_2] and two levels of soil moisture, 3 x 2 x 2 x 2 = 24), and [CO_2] and photoperiod for (a)-right, (b), (c) and (d) (n =48: three seedlings with two replications per treatment, two levels of [CO_2] and four levels of photoperiod, 3 x 2 x 2 x 4 = 48), because the 3-way interaction was not statistically significant. Means with different letters in figure 1(a) are significantly different from each other based on Fisher's Least Significant Difference post hoc test (p<0.05). Significance levels for each effect:*p<0.05; **p<0.01; ***p<0.001 were indicated on the top.

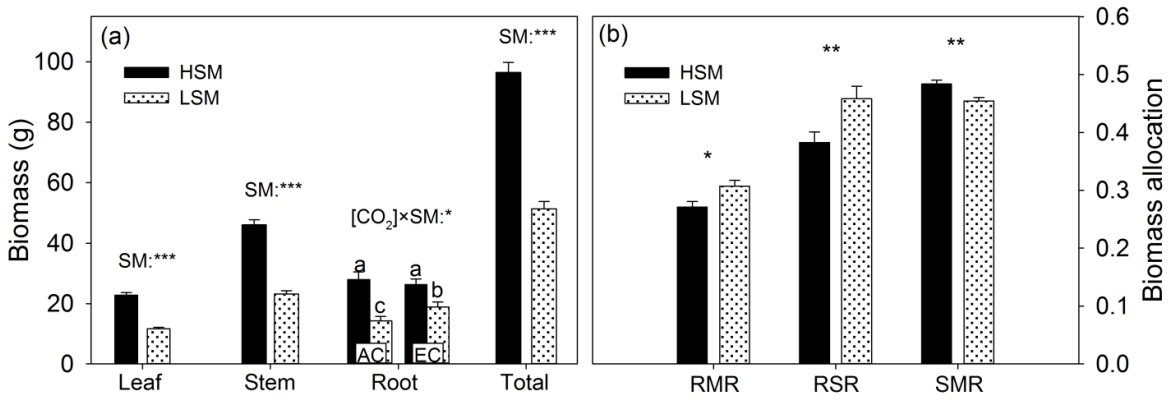

Figure 3.2 Mean (+SE) biomass (leaf, stem, root and total) (a) and biomass allocation (root mass ratio: RMR, root/ shoot ratio: RSR, and shoot mass ratio: SMR) (b) of trembling aspen seedlings grown under two levels of [CO_2] (ambient vs. elevated), and two levels of soil moisture (high vs. low) for two growing seasons. Data are pooled across [CO_2] and photoperiod for ((a)-except root biomass) and (b) (n =48 (see Figure 1 for explanations)), and data for root biomass in (a) are pooled across photoperiod (n =24, three seedlings with two replications per treatment, four levels of photoperiod, 3 x 2 x 4 = 24). The minuscule letters indicate the interactive effect of [CO_2] and soil moisture (SM). Means with different letters are significantly different from each other based on Fisher's Least Significant Difference post hoc test (p<0.05). Significance levels:*p<0.05; **p<0.01; ***p<0.001.

Foliar gas exchange and chlorophyll fluorescence measurements

The photoperiod treatment did not affect the net photosynthetic rate measured at treatment [CO_2] (P_n) in either measurement, whereas EC increased the August measurement of P_n only in LSM treatment. Interestingly, LSM treatment significantly increased P_n in both [CO_2] treatments in July but only in EC in August (Table 3.2, Fig. 3.3a). However, there were no significant interactive effects of soil moisture and photoperiod or of [CO_2], photoperiod, and soil moisture on any of the gas exchange parameters (Table 3.2). LSM significantly reduced stomatal conductance (g_s), and transpiration rate (E) in all photoperiod and [CO_2] treatments in July, but the magnitude of the effect on g_s declined with increasing photoperiod in August (Table 3.2, Fig. 3.3b & 3c). It is interesting to note that g_s showed a general decreasing trend with increasing growing-season photoperiod or latitude (from 48 to 58˚N) in seedlings grown under HSM, but

such a trend did not exist in seedlings grown under the LSM (Fig. 3.3b). LSM increased instantaneous water-use efficiency (iWUE*)* in both [CO_2] treatments, but the effect was greater under EC (Fig. 3.3d). EC increased iWUE in both soil moisture treatments in July, but the effect was not significant in the HSM in August (Table 2, Fig. 3.3d). LSM reduced the ratio of internal to ambient [CO_2] (C_i/C_a) in all photoperiod and [CO_2] treatments (Table 3.2, Fig. 3.3e).

The LSM treatment significantly increased the maximum rate of Rubisco carboxylation (V_{cmax}) and the maximum rate of electron transport for RuBP regeneration (J_{max}) in both July and August, but the magnitude of increase varied with photoperiod or latitude in August (Table 3.2, Fig. 3.4a & 4b). The August measurements of V_{cmax} and J_{max} showed a general increasing trend with increasing latitude or growing-season photoperiod in seedlings grown under LSM, but no such a trend was revealed in seedlings grown under HSM or in the July measurement of either moisture treatments (Fig. 3.4a &4b). However, the difference in V_{cmax} and J_{max} between two adjacent photoperiods was not always statistically significant (Fig. 3.4). LSM significantly increased J_{max}/V_{cmax} in July in both [CO_2] treatments, but only in AC in the August measurement (Fig. 3.4c). LSM significantly decreased the net photosynthetic rate measured at the ambient [CO_2] (P_{n400}) only in the 48˚N photoperiod in August, but no difference was found between soil moisture treatments in the photoperiods of higher latitudes (Fig.3.4d). Moreover, the August measurement of P_{n400} was marginally lower in EC than AC, indicating a photosynthetic downregulation in response to EC (Table 3.2). LSM significantly increased the quantum efficiency of photosystem II (F_v/F_m) (Table 3.2, Fig. 3.5). EC significantly reduced F_v/F_m in the photoperiod regimes associated with two lower latitudes but did not significantly affect it in the photoperiod regimes of the two higher latitudes (Fig. 3.5). However, there were no clear trends in the response of F_v/F_m to photoperiod regime (Fig. 3.5).

Table 3.2 ANOVA results (*p* values) for the effects of [CO_2] (C), photoperiod (P), soil moisture (SM) and their interactions on net photosynthesis at growth condition (P_n) and ambient [CO_2] (P_{n400}), stomatal conductance (*g*s), transpiration rate (*E*), instantaneous water-use efficiency (iWUE), maximal rate of Rubisco carboxylation (V_{cmax}), maximal RuBP regeneration rate (J_{max}), internal to ambient CO_2 concentration ratio (*C*i/*C*a) and the maximum quantum efficiency of PSII (F_v/F_m). The seedlings were exposed to two levels of [CO_2] (400 and 1000 µmol mol-1), four photoperiod regimes (at 48.38°N (seed origin), 51.68°N, 54.98°N, and 58.28°N), and two soil moisture regimes (high soil moisture (75%v to 45%v) and low soil moisture (20%v to 10%)). The numbers in bold are significant at <0.05.

Variable	Month	C (*DF=1)	P (DF=3)	SM (DF=1)	C × P (DF=3)	C × SM (DF=1)	P × SM (DF=3)	C × P × SM (DF=3)
P_n	*July*	0.171	0.817	**0.005**	0.458	0.305	0.404	0.991
	August	**0.014**	0.213	**0.002**	0.357	**0.001**	0.113	0.399
g_s	*July*	0.806	0.341	**0.001**	0.932	0.687	0.242	0.922
	August	0.495	0.343	**<0.001**	0.918	0.169	**0.046**	0.311
E	*July*	0.455	0.452	**<0.001**	0.821	0.496	0.357	0.976
	August	0.401	0.187	**<0.001**	0.311	0.880	0.271	0.687
iWUE	*July*	**0.002**	0.849	**<0.001**	0.438	**0.017**	0.063	0.664
	August	0.075	0.799	**<0.001**	0.604	**0.001**	0.677	0.991
V_{cmax}	*July*	0.792	0.368	**<0.001**	0.317	0.735	0.444	0.777
	August	0.092	**0.022**	**<0.001**	0.083	0.342	**0.004**	0.051
J_{max}	*July*	0.613	0.552	**<0.001**	0.448	0.835	0.304	0.608
	August	0.847	0.315	**<0.001**	0.302	0.577	**0.043**	0.289
J_{max}/V_{cmax}	*July*	0.201	0.410	**<0.001**	0.117	0.272	0.486	0.505
	August	0.137	0.186	**0.030**	0.328	**0.022**	0.761	0.551
P_{n400}	*July*	0.155	0.860	0.259	0.623	0.110	0.494	0.948
	August	0.060	**0.013**	0.265	0.176	0.120	**0.043**	0.213
C_i/C_a	*July*	0.138	0.799	**<0.001**	0.582	0.138	0.597	0.488
	August	0.114	0.513	**<0.001**	0.240	0.286	0.318	0.498
F_v/F_m	*August*	0.648	**0.015**	**<0.001**	**0.003**	0.168	0.258	0.988

*DF=degree of freedom

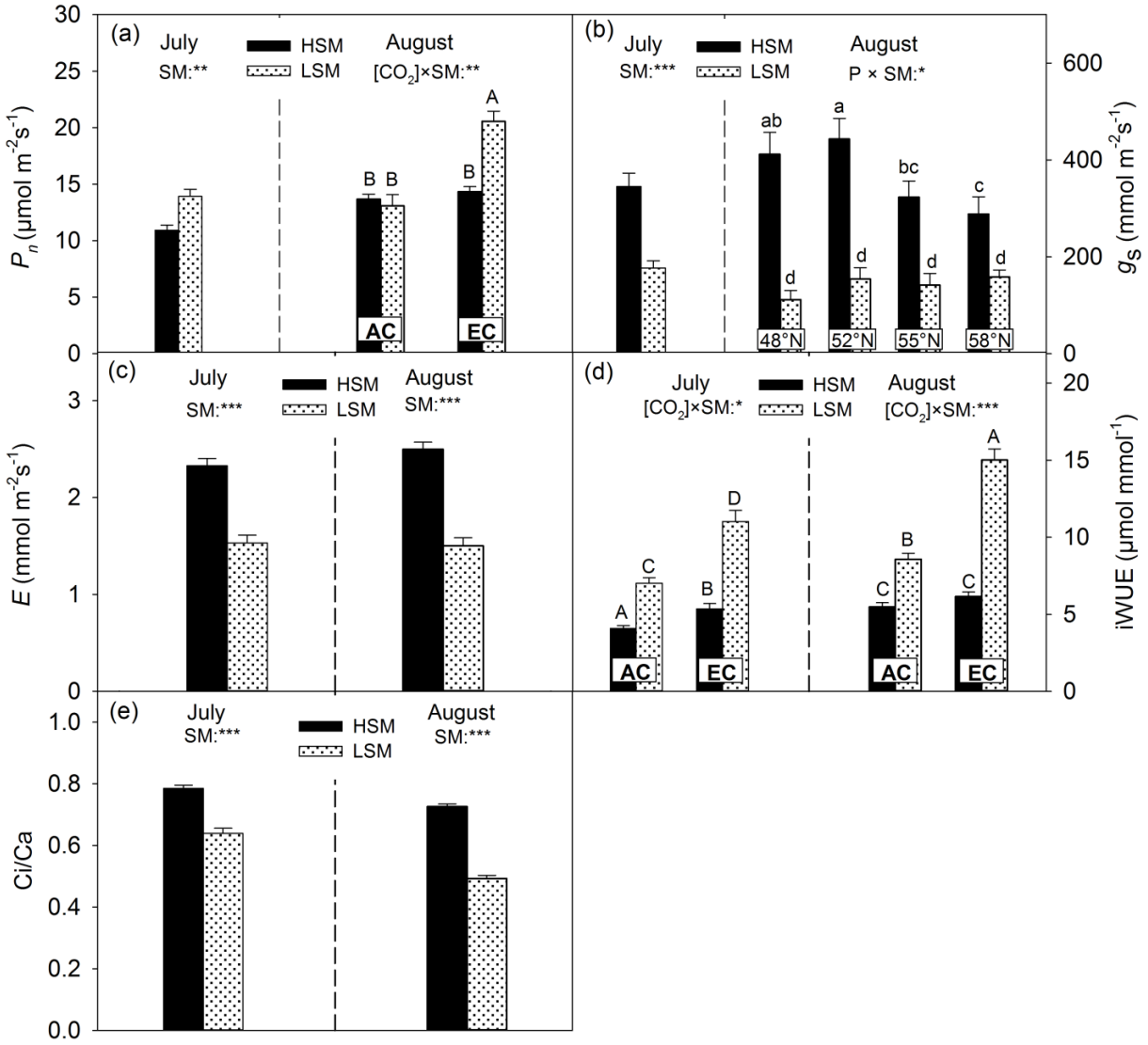

Figure 3.3 Mean (+SE) in situ rate of net photosynthesis (P_n) (a), stomatal conductance (g_s) (b), transpiration rate (E) (c), instantaneous water-use efficiency (iWUE) (d), and C_i/C_a of trembling aspen seedlings grown under two levels of [CO_2] (ambient vs. elevated), four photoperiod regimes (at 48 (seed origin), 52, 55, and 58°N) and two levels of soil moisture (high vs. low). Data are pooled across [CO_2] and photoperiod for (a)-left, (b)-left, (c), (e) (n =48 (see Figure 1 for explanations)), photoperiod for (a)-right, (d) (n =24 (see Figure 2 for explanations)), and [CO_2] for (b)-right (n =12: three seedlings with two replications per treatment, and two levels of [CO_2], 3 x 2 x 2 = 12). The measurements were carried out in July (left panel) and August (right panel). The minuscule letters indicate the interactive effect of photoperiod (P) × soil moisture (SM) and the majuscule letters indicate the interactive effect of [CO_2] × soil moisture (SM). Means with different letters are significantly different from each other based on Fisher's Least Significant Difference post hoc test ($p < 0.05$). Significance levels: *p<0.05; **p<0.01; ***p<0.001.

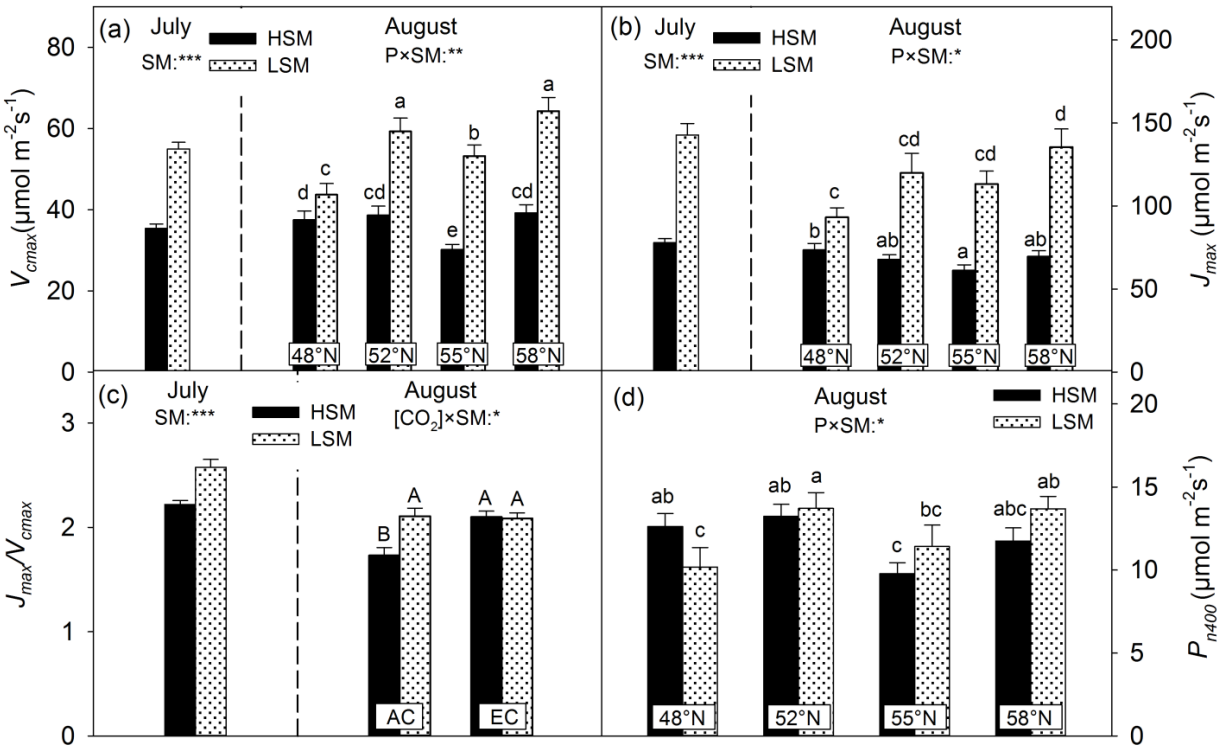

Figure 3.4 Mean (+SE) maximum rate of Rubisco carboxylation (V_{cmax}) (a), maximum rate of electron transport for RuBP regeneration (J_{max}) (b), J_{max} / V_{cmax} ratio (c) and net photosynthetic rate at ambient [CO_2] (P_{n400}) (d) in trembling aspen seedlings grown under two levels of [CO_2] (ambient vs. elevated), four photoperiod regimes (at 48(seed origin), 52, 55, and 58°N) and two levels of soil moisture (high vs. low), measured in July (left panel) and August (right panel). Data are pooled across [CO_2] and photoperiod for (a)-left, (b)-left, (c)-left (n =48(see Figure 1 for explanations)), [CO_2] for (a)-right (b)-right (n =12 (see Figure 3 for explanations)), photoperiod for (c-right) (n =24 (see Figure 1 for explanations)), and [CO_2] for (b)-right and (d)-right (n =12 (see Figure 3 for explanations)). The minuscule letters indicate the interactive effect of photoperiod × soil moisture (SM) and the majuscule letters indicate the interactive effect of [CO_2] × of soil moisture. Means with different letters are significantly different from each other based on Fisher's Least Significant Difference post hoc test (p<0.05). Significance levels: *p<0.05; **p<0.01; ***p<0.001.

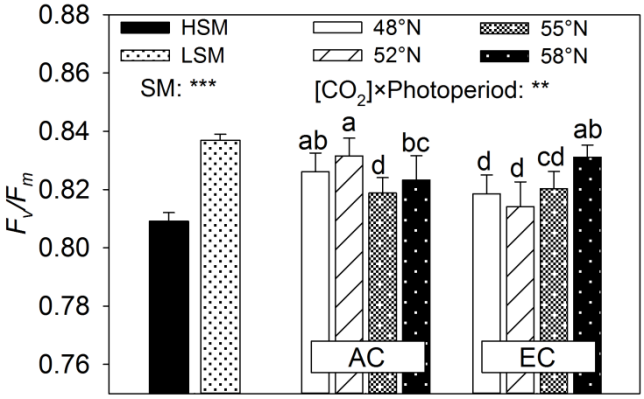

Figure 3.5 Quantum efficiency of photosystem II (F_v/F_m) (mean+ SE) of trembling aspen seedlings grown under two levels of [CO_2] (ambient vs. elevated), four photoperiod regimes (at 48(seed origin), 52, 55, and 58°N) and two levels of soil moisture (high vs. low) for two growing seasons. Data are pooled across [CO_2] and photoperiod for (left) (n =48 (see Figure 1 for explanations)), soil moisture for (right) (n =12, three seedlings with two replications per treatment, and two levels of soil moisture, 3 x 2 x 2 = 12). The minuscule letters indicate the interactive effect of [CO_2] × photoperiod. Means with different letters are significantly from each other based on Fisher's Least Significant Difference post hoc test (p<0.05). Significance levels: **p<0.01; ***p<0.001.

3.5 Discussion

The main effects of [CO_2] and photoperiod, and their interaction were not significant sources of variation for most of the growth and biomass variables. The lack of treatment effect on those variables was similar to the results of some other studies where trees were exposed to elevated [CO_2] under different photoperiods (Johnsen and Seiler 1996, Newaz et al. 2016, 2017). However, in the present study, the root biomass of water-stressed seedlings grown under the elevated [CO_2] was greater than that of seedlings that were water-stressed under the ambient [CO_2]. The consequent increase in water uptake capacity should ameliorate the negative effects of water stress (Wullschleger et al. 2002). With the predicted increase in the frequency and severity of water stress in many regions of North America (Pachauri et al. 2014), elevated [CO_2]-induced increases in root biomass might be beneficial to the northward migration of boreal trees

at dry sites, particularly for water stress-sensitive species like trembling aspen (*Populus tremuloides* Michx.) (Perala 1990; Peterson and Peterson 1992).

The height growth of trembling aspen seedlings generally increased with increasing photoperiod. This result is in agreement with other studies (Oleksyn et al. 1992, Johnsen and Seiler 1996). The higher height growth at longer photoperiod, particularly longest photoperiod at 58˚N latitude, was likely attributable to the longer time periods of photosynthesis as well as longer growing season due to delays in growth cessation (Farmer 1993). The longer hours of photosynthesis and increased efficiency of light energy conversion into biomass under a longer photoperiod regime have been reported as major contributors to the increased growth of white spruce (*Picea glauca* (Moench) Voss) (Stinziano and Way 2017). Indeed, the cessation of height growth occurred later in the longer photoperiod regime of 58˚N latitude than the shorter photoperiod regimes in this study (Inoue et al., unpublished). The rate of height growth is an important determinant for the survival of pioneer species and frequently used as an indicator of fitness (Ying and Yanchuk 2006). A higher rate of height growth during the juvenile stage would give a species advantages in its competition for resources, particularly light (Ying et al. 1985, Simard 1992). Trembling aspen is a fast-growing species that is very intolerant of shade (Burns et al. 1990) and having a faster rate of height growth than its competition species may be critical for winning the competition and surviving at new locations if it migrates. However, the stimulation of height growth by longer photoperiods may diminish as the canopy of the young forest closes (Merritt and Kohl 1982, 1983, Burdett and Yamamoto 1986). But this should not reduce the significance of the effect to dominate trees because they are not shaded by neighboring trees, and they are the future of the forest stand. However, what is interesting but unknown is whether the effects of photoperiods on height growth will decline as trees get older or bigger. Koch and Fredeen

(2005) have pointed out that increased height growth may increase trees' vulnerability to water stress because hydraulic resistance for water transport from roots to leaf increases with tree height.

My results indicate that morphological acclimation was the primary mechanism of acclimation to low soil moisture conditions in trembling aspen. The seedlings grown under the low soil moisture had a higher rate of photosynthesis than those grown under the high soil moisture condition and the effect varied with the season. The positive effect of low soil moisture occurred in both [CO_2] treatments in July, but only in the elevated [CO_2] in August. Furthermore, the elevated [CO_2] enhanced P_n only under the low soil moisture. The increase in P_n was accompanied by an increase in biomass allocation to roots. Presumably, the increased proportion of biomass allocated to roots reduced the level of moisture stress that the seedlings experienced internally because the same amount of leaf area was supported by a greater amount of roots. The combination of higher P_n and increased biomass allocation to roots resulted in much higher photosynthetic water use efficiency for seedlings grown under low soil moisture, particularly under elevated [CO_2]. Trembling aspen generally displays a poor ability to reduce the impact of drought by regulating stomatal conductance (Dang et al. 1997). Therefore, it is reasonable to conclude that morphological acclimation is the primary and effective mechanism in the response of trembling aspen to drought stress (Dang et al. 1997). However, it should be pointed out that a higher photosynthetic rate does not necessarily translate into a higher carbohydrate production. The lack of correlation between the whole plant productivity and leaf photosynthetic rate per leaf area is common (Evans 1998). For example, increases in leaf-level photosynthetic rate may often be achieved by increased investment of nitrogen per unit leaf area (Beadle and Long 1985). However, morphological acclimation in response to drought stress may also involve a reduction

in leaf area, and the product of photosynthetic rate and total leaf area determines the total gross production of carbohydrates.

It is interesting to note that the magnitude of g_s reduction by the low soil moisture declined with increasing photoperiod (in the August measurement) and this trend was accompanied by the negative effect of longer photoperiods on g_s. This interactive effect suggests that the difference in the productivity of trembling aspen grown on sites with different soil moisture conditions may become smaller if the species migrate to higher latitudes. The most likely reason for the reduction of g_s with increasing photoperiod is likely the daily duration of transpiration. The primary control of water loss from plants is the aperture of stomates. In most C_3 plants, stomates normally remain at least partially open during the day and close in the darkness of night to re-hydrate when CO_2 is not required for photosynthesis (Tallman 2004). The longer the photoperiod is, the longer the stomates will remain open and the more water will be lost. The water potential is generally the more negative during the day and the tissues get recharged with water at night. If the night is too short or photoperiod is too long, the tissues may not have enough time to re-hydrate themselves because starting to lose water again the next day. Consequently, plants growing under a long photoperiod may suffer from physiological drought stress even when there is plenty of water in the soil. Indeed, the stomatal conductance in the high soil moisture treatment declined with increasing photoperiod in this study.

My results suggest that the increase of photosynthesis in trembling aspen grown under low soil moisture was associated with an upregulation of photosynthetic apparatus. The seedlings grown under the low soil moisture showed significantly higher V_{cmax}, $J_{max,}$ and F_v/F_m than those grown under the high soil moisture. Further, the low soil moisture increased V_{cmax} in longer

photoperiods. This result is contrary to my expectation that the longer photoperiod would exacerbate the negative impact of low soil moisture. There are various strategies that plants use in their response to stresses, and some strategies involve shifts in leaf and whole plant physiology, structure, and nitrogen allocation (e.g., Zhou et al. 2016, Zait et al. 2019, Panković et al. 1999). Acclimation to prolonged low moisture has been associated with an increase in foliar nitrogen concentration in various species (e.g., Reich et al. 1999, Panković et al. 1999, Wright et al. 2001, Wright and Westoby 2002, Kitao et al. 2003, Aranda et al. 2005). Panković et al. (1999) have reported a substantial increase in the concentrations of nitrogen and soluble proteins and in Rubisco activity to counteract the negative effect of prolonged moisture stress. These increases can lead to positive photosynthetic acclimation (Kitaoka and Koike 2005). Increases in biomass allocation to roots, reductions in biomass allocation to leaves, and the upregulation photosynthetic apparatus represent a feedforward strategy in response to low soil moisture.

In conclusion, since more frequent and longer periods of water stress are predicted in many regions of North America in the future (Pachauri, Allen, Barros, Broome, Cramer, et al. 2014), trees will need effective strategies to cope with water stress, which may hinder their establishment and slow-down their migration in response to climate change. My results reveal that morphological acclimation is likely the primary mechanism in the response of trembling aspen to low soil moisture, resulting in the counterintuitive physiological responses to water stress. Such physiological responses are further magnified by elevated [CO_2] and longer photoperiod associated with northward migration. This paper highlights the need to contemplate the interactive effects of [CO_2], photoperiod, and soil moisture when planning assisted migration or predicting shifts in the distribution of boreal forests in the future.

3.6 Reference

Aranda I, Castro L, Pardos M, Gil L, Pardos JA (2005) Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quercus suber* L.) seedlings. For Ecol Manag 210:117–129.

Beadle CL, Long SP (1985) Photosynthesis — is it limiting to biomass production? Biomass 8:119–168.

Burdett AN, Yamamoto S (1986) Growth rate and shoot: Root allometry in *Pseudotsuga menziesii* (Mirb.) Franco and Pinus contorta Dougl. Seedlings raised under two photoperiodic regimes. Scand J For Res 1:397–402.

Burns RM, Honkala BH, Coordinators T (1990) Silvics of North America: Volume 2. Hardwoods. U S Dep Agric USDA For Serv Agric Handb 654

Comstock J, Ehleringer JR (1986) Photoperiod and photosynthetic capacity in *Lotus scoparius*. Plant Cell Environ 9:609–612.

Craine JM (2009) Resource Strategies of Wild Plants. Princeton University Press.

Dang Q-L, Margolis HA, Coyea MR, Sy M, Collatz GJ (1997) Regulation of branch-level gas exchange of boreal trees: roles of shoot water potential and vapor pressure difference. Tree Physiol 17:521–535.

Delbart N, Picard G, Le Toan T, Kergoat L, Quegan S, Woodward IAN, Dye D, Fedotova V (2008) Spring phenology in boreal Eurasia over a near century time scale. Glob Change Biol 14:603–614.

Evans LT (1998) Greater crop production. Oxford University Press Cary, NC, USA.

Farmer RE (1993) Latitudinal variation in height and phenology of balsam poplar. Silvae Genet 42:148–148.

Hay RKM (1990) The influence of photoperiod on the dry matter production of grasses and cereals. New Phytol 116:233–254.

Hertel D, Strecker T, Müller-Haubold H, Leuschner C (2013) Fine root biomass and dynamics in beech forests across a precipitation gradient - is optimal resource partitioning theory applicable to water-limited mature trees? Guo D (ed). J Ecol 101:1183–1200.

Hogg EH, Brandt JP, Kochtubajda B (2002) Growth and dieback of aspen forests in northwestern Alberta, Canada, in relation to climate and insects. Can J For Res 32:823–832.

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Can J For Res 38:1373–1384.

Huang J-G, Bergeron Y, Denneler B, Berninger F, Tardif J (2007) Response of forest trees to increased atmospheric CO_2. Crit Rev Plant Sci 26:265–283.

Jach ME, Ceulemans R, Murray MB (2001) Impacts of greenhouse gases on the phenology of forest trees. Impact Carbon Dioxide Greenh Gases For Ecosyst CAB Int Wallingford:193–235.

Johnsen KH, Seiler JR (1996) Growth, shoot phenology and physiology of diverse seed sources of black spruce: I. Seedling responses to varied atmospheric CO_2 concentrations and photoperiods. Tree Physiol 16:367–373.

Keeling CD, Chin JFS, Whorf TP (1996) Increased activity of northern vegetation inferred from atmospheric CO_2 measurements. Nature 382:146–149.

Kitao M, Lei TT, Koike T, Tobita H, Maruyama Y (2003) Higher electron transport rate observed at low intercellular CO_2 concentration in long-term drought-acclimated leaves of Japanese mountain birch (*Betula ermanii*). Physiol Plant 118:406–413.

Kitaoka S, Koike T (2005) Seasonal and yearly variations in light use and nitrogen use by seedlings of four deciduous broad-leaved tree species invading larch plantations. Tree Physiol 25:467–475.

Koch GW, Fredeen AL (2005) Transport challenges in tall trees. In: Holbrook NM, Zwieniecki MA (eds) Vascular transport in plants. Academic Press, Burlington, pp 437–456.

Lafleur B, Pare D, Munson AD, Bergeron Y (2010) Response of northeastern North American forests to climate change: Will soil conditions constrain tree species migration? Environ Rev 18:279–289.

Marchetti SB, Worrall JJ, Eager T (2011) Secondary insects and diseases contribute to sudden aspen decline in southwestern Colorado, USA. Can J For Res 41:2315–2325.

McKenney DW, Pedlar JH, Lawrence K, Campbell K, Hutchinson MF (2007) Potential Impacts of Climate Change on the Distribution of North American Trees. BioScience 57:939–948.

McKenney DW, Pedlar JH, Rood RB, Price D (2011) Revisiting projected shifts in the climate envelopes of North American trees using updated general circulation models. Glob Change Biol 17:2720–2730.

Merritt RH, Kohl Jr HC (1982) Effect of root temperature and photoperiod on growth and crop productivity efficiency of petunia [Alternate heat sources, use of energy]. J-Am Soc Hortic Sci USA

Merritt RH, Kohl Jr HC (1983) Crop productivity efficiency of petunias in the greenhouse. J Am Soc Hortic Sci

Moench RD (1999) Aspen Seed Collection. Nursery Information Series Colorado State Forest Service, Fort Collins CO.

Myneni RB, Keeling CD, Tucker CJ, Asrar G, Nemani RR (1997) Increased plant growth in the northern high latitudes from 1981 to 1991. Nature 386:698.

Nelson RJ, Denlinger DL, Somers DE (2010) Photoperiodism: the biological calendar. Oxford University Press.

Newaz MS, Dang Q-L, Man R (2016) Morphological response of jack pine to the interactive effects of carbon dioxide, soil temperature and photoperiod. Am J Plant Sci 7:879.

Newaz S, Dang Q-L, Man R (2017) Eco-physiological potential of jack pine (Pinus banksiana) for assisted northward migration: interactions among photoperiod, [CO_2] and moisture stress. Nord J Bot 35:633–643.

Oleksyn J, Tjoelker MG, Reich PB (1992) Growth and biomass partitioning of populations of european pinus sylvestris l. under simulated 50∘ and 60∘ N daylengths: evidence for photoperiodic ecotypes. New Phytol 120:561–574.

Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe QD, Dasqupta P (2014) Climate change 2014 synthesis report. contribution of working groups I, II, and III to the fifth assessment report of the Intergovernmental Panel on Climate Change.

Panković D, Sakač Z, Kevrešan S, Plesničar M (1999) Acclimation to long-term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. J Exp Bot 50:128–138.

Passioura JB (1996) Drought and drought tolerance. Plant Growth Regul 20:79–83.

Perala DA (1990) *Populus tremuloicies* Michx.-Quaking Aspen. Silv N Am Hardwoods Burns RM Honkala BH Eds:555–569.

Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. Funct Plant Biol 27:1191–1191.

Poorter H, Niklas KJ, Reich PB, Oleksyn J, Poot P, Mommer L (2012) Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. New Phytol 193:30–50.

Reich PB, Ellsworth DS, Walters MB, Vose JM, Gresham C, Volin JC, Bowman WD (1999) Generality of leaf trait relationships: a test across six biomes. Ecology 80:1955–1969.

Robredo A, Pérez-López U, de la Maza HS, González-Moro B, Lacuesta M, Mena-Petite A, Munoz-Rueda A (2007) Elevated CO_2 alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. Environ Exp Bot 59:252–263.

Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139:395–436.

Seager R, Neelin D, Simpson I, Liu H, Henderson N, Shaw T, Kushnir Y, Ting M, Cook B (2014) Dynamical and Thermodynamical Causes of Large-Scale Changes in the Hydrological Cycle over North America in Response to Global Warming. J Clim 27:7921–7948.

Sherwood S, Fu Q (2014) A Drier Future? Science 343:737–739.

Simard S (1992) Ecology and management of paper birch and black cottonwood in southern British Columbia. B.C. Ministry of Forests, Victoria, B.C.

Stulen I, Den Hertog J (1993) Root growth and functioning under atmospheric CO_2 enrichment. Vegetatio 104:99–115.

Tallman G (2004) Are diurnal patterns of stomatal movement the result of alternating metabolism of endogenous guard cell ABA and accumulation of ABA delivered to the apoplast around guard cells by transpiration? J Exp Bot 55:1963–1976.

Thomas B, Vince-Prue D (1996) Photoperiodism in plants. Elsevier.

Wang Y, Hogg EH, Price DT, Edwards J, Williamson T (2014) Past and projected future changes in moisture conditions in the Canadian boreal forest. For Chron 90:678–691.

Worrall JJ, Rehfeldt GE, Hamann A, Hogg EH, Marchetti SB, Michaelian M, Gray LK (2013) Recent declines of *Populus tremuloides* in North America linked to climate. For Ecol Manag 299:35–51.

Wright IJ, Reich PB, Westoby M (2001) Strategy shifts in leaf physiology, structure and nutrient content between species of high-and low-rainfall and high-and low-nutrient habitats. Funct Ecol 15:423–434.

Wright IJ, Westoby M (2002) Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. New Phytol 155:403–416.

Wullschleger SD, Tschaplinski TJ, Norby RJ (2002) Plant water relations at elevated CO_2 -- implications for water-limited environments. Plant Cell Environ 25:319–331.

Ying CC, Illingworth K, Carlson M (1985) Geographic variation in lodgepole pine and its implications for tree improvement in British Columbia. In: Lodge pine-the specis and its maanagement, Spokane, Washington (USA); Vancouver, British Columbia (Canada), 8-16 May 1984. Washington State University, Cooperative Extension

Ying CC, Yanchuk AD (2006) The development of British Columbia's tree seed transfer guidelines: Purpose, concept, methodology, and implementation. For Ecol Manag 227:1–13.

4 Chapter 4: Photoperiod, [CO_2] and soil moisture interactively affect the phenology and growing season length of trembling aspen: a perspective for climate change-induced tree migration

4.1 Abstract

Trees may migrate to higher latitudes in response to climate changes, which would expose them to different environmental conditions, such as [CO_2], photoperiod, and soil moisture regimes. Changes in [CO_2], photoperiod, and soil moisture regimes will likely influence the phenology and growth in trees. I examined the effects of [CO_2] (AC: 400 and EC: 1000 μmol mol^{-1}), photoperiod (corresponding to 48°N (seed origin), 52°N, 55°N, and 58°N latitude), soil moisture regimes (high soil moisture (HSM): 60%-75% and low soil moisture (LSM): 13%-20% of field capacity) and their interactions on the phenology of in trembling aspen (*Populus tremuloides* Michx.) for two growing seasons. I found that longer photoperiods delayed growth cessation, bud set, leaf senescence, extending the growing season and increasing height growth. LSM also delayed autumnal phenology, but reduced growth and the magnitudes of the effects were reduced by elevated [CO_2]. Surprisingly, LSM also reduced seedling cold hardiness in the fall. EC delayed bud break but expedited leaf unfolding in the spring, and the effects were magnified by LSM. Significant interactions between photoperiod, [CO_2] and soil moisture, as evidenced by their impact on phenology, cold hardiness, growth and the length of the growing season, indicate the complex nature of latitudinal tree migration that has been predicated under the scenario of climate change. Thus, it is critically important that these complicated interactions are considered when planning latitudinal seed transfers and predicting the natural migration of boreal trees in response to climate change.

Keywords: tree migration; photoperiod; [CO_2]; soil moisture: *Populus tremuloides* Michx.; growth cessation; bud set; leaf senescence; cold hardiness; bud break.

4.2 Introduction

Rising atmospheric CO_2 concentration ([CO_2]) increases temperatures and changes precipitation patterns (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014), leading to the possible northward migration of tree species (McKenney et al. 2007). Migrating toward the north, however, will expose trees to different photoperiod regimes from their current location, e.g., longer photoperiods during the growing season and faster rates of changes in photoperiod during seasonal transitions (Thomas and Vince-Prue 1997). These will likely alter the timing of phenological events, which are major determinants of tree growth and species distribution (Chuine and Beaubien 2001, Chuine 2010, Way and Montgomery 2015). Also, climate change alters the availability of [CO_2] and soil moisture (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). Past studies, however, have primarily focused on the individual effects of these factors (e.g., Leuzinger, Zotz, Asshoff, & Körner, 2005; Taylor et al., 2008). While a good understanding of individual effects is essential, changes in these factors will more likely interact with each other, and the interactive effects may be different from the summation of individual effects. This study investigated the interactive effects of photoperiod regime, [CO_2] and soil moisture on the phenology of boreal trembling aspen in the context of climate-induced northward migration.

Photoperiod is one of the critical environmental factors regulating seasonal changes in phenology, physiology, and growth of temperate and boreal plants (Weiser 1970, Junttila 1980, Jackson 2009). Phenological events in the fall (e.g., growth cessation, bud set, leaf senescence,

cold acclimation) are induced mainly by longer nights and lower temperatures (Weiser 1970, Jackson 2009, Fracheboud et al. 2009, Soolanayakanahally et al. 2013). These phenological events are generally related to the adaption of the species to the local environment, for example, northern populations set bud earlier and acquire higher cold hardiness than southern populations (Campbell and Sorensen 1973, Junttila 1982, Kozlowski and Pallardy 2002). Thus, by moving trees north out of their current location, trees may experience a mismatch between their phenology and the environment at the new site. For instance, growth cessation may be delayed, and the risk of frost damage may increase in early autumn (Howe, Hackett, Furnier, & Klevorn, 1995). Further, the faster rate of photoperiod change during the summer-autumn transition at higher latitudes may jeopardize the proper development of cold hardiness and buds (Bigras et al. 2001). Trees without an adequate level of cold hardiness may not survive cold winters (Welling et al. 1997, Li et al. 2003), or the frost-damaged buds may not be able to flush in the following spring (Ostry et al. 1989). On the other hand, the delay of autumnal phenology could be beneficial to trees in that the time period for photosynthetic carbohydrate production will be longer, enhancing metabolic processes and nutrient resorption of leaves during leaf senescence (Hoch et al. 2001) and benefiting bud break next spring (Kaelke and Dawson 2005, Regier et al. 2010). Therefore, a good understanding of how photoperiod regimes at higher latitudes may affect tree growth, dormancy induction, and cold hardiness development may be critical for predicting the potential performance of trees at new locations in the context of climate change-induced northward migration.

Elevated [CO_2] and soil moisture can also affect tree phenology and cold hardiness, and the effects can vary with species and other environmental factors (Gunderson et al. 1993). Higher [CO_2] has been reported to expedite bud burst in the spring and advance leaf senescence in the

fall in some species (Jach and Ceulemans 1999), delay leaf senescence in other species (Tricker et al. 2004, Taylor et al. 2008, Riikonen et al. 2008), or have no effect on their traits (Herrick and Thomas 2003). The interactions among different factors can complicate the prediction of trees' responses and performance when several factors simultaneously. For instance, the effects of low soil moisture on the phenology of trees in the fall (Holland, Koller, Lukas, & Brüggemann, 2016; Winner & Pell, 2012) and the development of cold hardiness (Kreyling et al. 2012) can be modified by changes in photoperiod and elevated [CO_2]. However, the interactive effects of multiple factors are generally not well understood.

Trembling aspen (*Populus tremuloides* Michx.) is the most abundant deciduous tree species in Canada's boreal forest and has important ecological and commercial values. *Populus* species are sensitive to photoperiod, and a shortening of photoperiod can trigger the initiation of autumnal phenological events (Pauley and Perry 1954, Keskitalo et al. 2005). Trembling aspen is also sensitive to water stress (Peterson and Peterson 1992); recent severe droughts, in combination with other stress factors such as insects and pathogens, have resulted in large areas of trembling aspen decline and die-back in west-central Canada (Hogg, Brandt, & Michaelian, 2008; Marchetti, Worrall, & Eager, 2011; Worrall et al., 2013). The high sensitivity of trembling aspen forests to drought causes serious concerns for the future of deciduous boreal forests, as rising temperature likely leads to drier climates (Hogg & Hurdle, 1995; Hogg & Bernier, 2005).

The main objective of this study was to investigate the phenotypic plasticity of trembling aspen in response to the interactions of [CO_2], photoperiod, and soil moisture regimes in the context of climate change-induced northward migration. I hypothesize that the changes in photoperiod regime associated with northward migration and elevated [CO_2] would delay the autumn

phenological events of trembling aspen, resulting in a longer growing season and more growth, and that low soil moisture would partially offset the delay in the development of dormancy and cold hardiness. I also hypothesize that photoperiod regimes at latitudes north of the seed origin in combination with elevated [CO_2] would expedite bud break in the spring.

4.3 Materials and Methods

4.3.1 Plant materials

Trembling aspen (*Populus tremuloides* Michx.) catkins were collected on June 9th, 2016 from 10 free-pollinated trees in Thunder Bay, Ontario, Canada (48.38°N, 89.25°W). The catkins were air-dried for five days at room temperature, and seeds were extracted according to Moench (1999). The seeds from different trees were mixed and stored in a sealed glass bottle at -4°C.

Seeds were sown in germination trays filled with 1:1 (v/v) mixture of peat moss and vermiculite in a greenhouse at Lakehead University Thunder Bay campus. The day/night temperatures in the greenhouse were maintained at 24/14 (±2) °C, and the natural photoperiod was extended to 16h by using high-pressure sodium lamps (P.L. Systems, Grimsby, ON, Canada). All trays were well-watered but not fertilized during the germination phase. The seedlings were individually transplanted into pots (12 cm in height, and 12 cm and 9.5 cm in the top and bottom diameter, respectively) filled with a mixture of peat moss and vermiculite (7:3 v/v) 18 days after the completion of germination.

4.3.2 Experimental design

The experiment was carried out in four greenhouses at Lakehead University, Thunder Bay campus. The treatments consist of two levels of [CO_2] (400 μmol mol^{-1} (AC-ambient [CO_2]) and

1000 µmol mol^{-1} (EC-elevated [CO_2]) µmol mol^{-1}), four photoperiod regimes corresponding to the photoperiod of 48.38°N (seed origin) (48°N), 51.68°N (52°N), 54.98°N (55°N), and 58.28°N (58°N) latitude, and two soil moisture regimes (high soil moisture - HSM and low soil moisture - LSM). The experiment design was a split-split plot with the [CO_2] as the main plot, photoperiod as sub-plot, and soil moisture as sub-sub-plot. Each of the two [CO_2] treatments were randomly assigned to two of the four greenhouses (2 replicates for each [CO_2]). The photoperiod treatment was nested within the [CO_2] treatment, and soil moisture was nested within the photoperiod.

4.3.3 Treatments and greenhouse environment controls

The environmental conditions in each greenhouse were independently monitored and controlled using the Argus Titan Environment Control System (Argus Controls Systems Ltd., Surrey, BC, Canada). The EC was achieved with the model GEN2E gas CO_2 generators made by Custom Automated Products Inc. (Riverside, California, USA). The photoperiod in each greenhouse was set to the longest photoperiod of the four treatments: the natural light level was supplemented (on cloudy days, early mornings, and late evenings) and natural day length was lengthened by using high-pressure sodium lamps (Model LR48877, P.L. Systems, Grimspy, ON, Canada). The shorter photoperiods were achieved by shading. A wooden frame was installed for each photoperiod treatment, and neutral density shading cloth was used to block light in the shorter photoperiod treatments. The photoperiod setting was adjusted weekly according to the calculated photoperiod for each of the four latitudes. The soil volumetric water content was monitored with Delta-T ML2x probe and HH2 moisture meter (Delta-T Devices, Cambridge, UK) and maintained between 60% and 75% of the field capacity for HSM and between 13% and 20% of the field capacity for LSM.

The experiment was run for two growing seasons, November 1st, 2016 to April 16th, 2017 (emulating the natural environmental conditions of June 7th to November 20th) and November 1st, 2017 to May 15th, 2018 (emulating the natural environmental conditions of April 26th to November 7th). The emulated natural dates were used in the subsequent presentations of the paper. The temperature and photoperiod were adjusted weekly based on the weekly averages of the past ten years for each of the four latitudes (Environment Canada weather records, ON). The daily temperature in each greenhouse was ramped at 6-hour set points (averages of the past ten years). The fertilizer solution contained 33 mg N L^{-1}, 73 mg P L^{-1} and 55 mg K L^{-1} in the establishment/early season phase (June 7 – 21 in the first growing season (first) and April 26 – May 25 in the second growing season (second)); 100 mg N L^{-1}, 60 mg P L^{-1}, 150 mg K L^{-1}, 80 mg Ca L^{-1}, 40 mg Mg L^{-1} and 60 mg S L^{-1} in the rapid growth phase (June 22 to August 30 in the first growing season May 26 to August 30 in the second growing season); and mg N L^{-1}, 44 mg P L^{-1} and 83 mg K L^{-1} in the hardening phase (September 1 - 25) (Landis 1989, Jacobs and Landis 2009). After the completion of bud set and cold hardening in the first growing season, the seedlings were stored in walk-in cold storage at -4°C. The seedlings were covered with plastic sheets to avoid freeze-desiccation during the storage. The seedlings were transferred into bigger pots (18 cm in height, and 16 cm and 14 cm in top/bottom diameter) in the second growing season.

4.3.4 Measurements and observations

Growth and growth cessation

Three seedlings were randomly selected from each treatment combination in each replication for weekly growth measurements. The weekly measurement of stem elongation started on Julian day 225 and continued until the completion of the elongation (6 weeks). The height growth

measurements were used to develop the height growth curve for each sample tree, which was used to determine the timing of growth cessation. According to MacLachlan *et al.* (2017), the date of growth cessation was defined as the date when 95% of annual height increment was achieved, and the percentage height growth was calculated as follows:

$$\text{Height growth (\%)} = \frac{\text{cumulative height increment (cm)}}{\text{Total height increment (cm)}} \times 100$$

The height growth curve was fitted using a 3-parameter sigmoidal model in SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). Due to time constraints, I only obtained height growth curve data in the first growing season.

Autumn bud phenology

The autumn bud phenology was observed at 2-day intervals from August (emulated dates) to the completion of bud set in the first and second growing season. The development of terminal buds was monitored and recorded using four scales: stage 0 = no bud formation (Figure 4.1a), stage 1= appearance of terminal bud (Figure 4.1b), stage 2 = terminal bud in light brown color (Figure 4.1c), and stage 3 = terminal bud fully developed and covered by dark brown scales (referred to as bud set hereafter) (Figure 4.1d).

Figure 4.1 A four-stage scale was used for monitoring autumn bud development. Picture (a) stage 0; no bud set (active growth phase), (b) stage 1; initiation of a terminal bud, (c) stage 2; terminal bud becoming light brown, and (d) stage 3; terminal bud fully developed and covered by dark brown scales (bud set).

Leaf senescence

Leaf senescence was observed at 4-day intervals starting from August until all leaves turned yellow in both growing seasons. Leaf color change and the number of leaves that turned yellow were assessed visually. The dates of 10% (initiation) and 100% (completion) leaf senescence for individual seedlings were determined from the data.

Electrolyte leakage test

The electrolyte leakage method was used to determine the cold hardiness after the seedlings were stored at -4°C for a month at the end of each of the two growing seasons (corresponding to mid-December of the natural season). The same seedlings were used for the phenological observations and cold hardiness determination. The tip of the terminal shoot from each seedling was cut into four 2-cm long sections and washed with distilled water (DW). Each section was put into a separate 50 ml falcon tube and stored at 4°C for 24h. Then, the samples were exposed to -5°C, -15°C, -30°C and -45°C using a programmable freezer (Model: 45-6.8, Scientemp Corporation, Adrian, MI, USA). The freezer temperature was lowered at a constant rate of 5 °C

h^{-1} (starting at 5 °C) and held for 1h after each 5 °C change. The samples were maintained at each target temperature for 1h before they were taken out of the freezer, placed in falcon tubes with 20 ml of DW and incubated for 24 hours at room temperature (21°C). The samples were well shaken before the initial electrical conductivity of the solution was measured using an Accumet AR 20 electrical conductivity meter (Fisher Scientific, Ottawa, Canada). The falcon tubes were then heated in a dry oven at 80°C for 2 hours, and the second measurement of electrical conductivity was taken after 24 hours at room temperature. The relative electrolyte leakage (REL) was calculated as follows (Zhang and Willison 1987);

$$\mathrm{REL}(\%)=\left(\frac{\text{initial conductivity}}{\text{second conductivity}}\right)\times 100\%$$

LT_{50} (the temperature causing 50% REL) was estimated from a linear regression of REL with the target freezing temperatures (Arora et al. 1992, Boorse et al. 1998, Rapacz 2002, Morin et al. 2007). For the ease of graphic presentation, the absolute value of LT_{50} was used in figure presentations

Spring bud phenology

The observation of spring phenology was conducted in the spring of the second growing season. Three seedlings from each treatment combination and replication were monitored at 2-day intervals using a five-stage scale: stage 0 = dormant (Figure 4.2a), stage1= bud open with a visible green tip (Figure 4.2b) (referred to as bud break hereafter), stage 2= large visible green tip shown (Figure 4.2c), stage 3 = green leaf emerged from bud scales with leaf bases hidden (Figure 4.2d) and stage 4 = leaf unfolding with very small leaves and visible petiole (Figure 4.2e). The monitoring started when seedlings were moved back into the greenhouses and ended

when all seedlings reached the leaf unfolding stage. Leaf unfolding time was calculated as the duration days from stage 1 to stage 4.

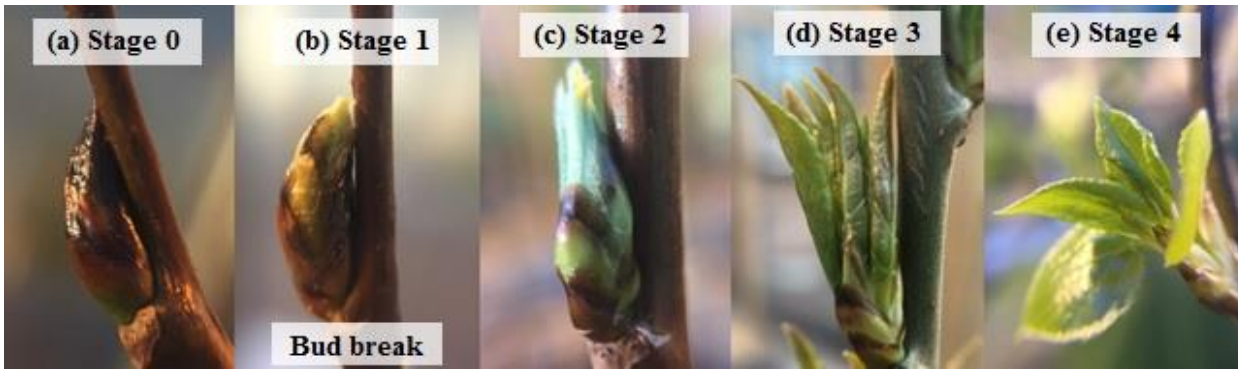

Figure 4.2 A five-stage scale was used for monitoring spring bud phenology. Picture (a) stage 0; dormant bud, (b) stage 1; bud open with visible green tip (bud break), (c) stage 2; large visible green tip shown, (d) stage 3; green leaf emerged from the bud with leaf bases hidden, and (e) stage 4; leaf unfolding with very small leaves and visible petiole.

4.3.5 Data Analysis

The data were examined graphically for the normality of distribution (probability plots of residuals) and homogeneity of variance (scatter plots) using the R software (Version 3.5.0, R Development Core Team 2018) before being subjected to the analysis of variance (ANOVA). An effect was considered significant at $p \leq 0.05$ and marginally significant at $p \leq 0.1$. When the ANOVA showed a significant ($p \leq 0.05$) photoperiod effect or significant interaction, Fisher's Least Significant Difference post hoc test was used to compare treatment means.

4.4 Results

Growth cessation and height growth

Growth cessation of seedlings grown in longer photoperiods corresponding to 55 and 58°N latitude was delayed by 5 and 6 days, respectively, as compared to 48°N photoperiod (Table 4.1;

Figure 4.3a). Low soil moisture (LSM) delayed growth cessation by 2 days (Table 4.1; Figure 4.3b). Total height growth showed a general increasing trend with increasing photoperiod, but not all the differences between adjacent treatments were statistically significant (Figure 4.4). LSM reduced height growth by about 12 cm (Table 4.1; Figure 4.4).

Table 4.1 ANOVA results (*p* values) for the effects of [CO_2] (C), photoperiod (P), soil moisture (SM) and their interactions on height increment, growth cessation, final height growth, autumn bud phenology, leaf senescence, spring bud phenology, and cold hardiness. The seedlings were exposed to two levels of [CO_2] (400 and 1000 μmol mol^{-1}), four photoperiod regimes (at 48°N (seed origin), 52°N, 55°N, and 58°N), and two soil moisture regimes (high (75%v to 45%v) and low (20%v to 10%)). The numbers in bold are at <0.1.

Variable	Year	C (*DF=1)	P (DF=3)	SM (DF=1)	C × P (DF=3)	C × SM (DF=1)	P × SM (DF=3)	C × P × SM (DF=3)
Growth cessation	*2017*	0.672	**0.047**	**0.012**	0.808	0.777	0.309	0.544
Final height	*2017*	0.202	**0.035**	**0.002**	0.718	0.247	0.558	0.661
Autumn bud phenology								
Bud set	*2017*	0.984	0.137	0.818	**0.017**	0.251	0.648	0.930
	2018	0.444	**0.028**	**<0.001**	0.171	0.911	0.538	0.897
Leaf senescence								
Initiation	*2017*	0.324	0.054	0.084	0.181	0.107	0.575	0.748
	2018	**0.010**	0.235	**<0.001**	0.814	**0.008**	0.051	0.424
Duration	*2017*	0.356	0.217	0.229	0.622	0.573	0.215	0.494
	2018	**0.036**	0.574	**0.001**	0.772	**0.009**	0.075	0.456
Completion	*2017*	0.926	0.056	0.153	0.127	0.070	0.318	0.281
	2018	0.159	0.116	**0.001**	0.892	0.477	0.666	0.879
Cold Hardiness								
LT50	*2017*	0.535	**0.003**	0.397	**0.042**	0.501	0.992	0.876
	2018	0.343	0.316	**<0.001**	0.875	0.455	0.946	0.740
Spring bud phenology								
Bud break	*2018*	**0.038**	0.611	0.055	0.622	**0.024**	0.459	0.264
Leaf unfolding	*2018*	0.067	0.275	0.797	0.112	**0.009**	0.168	0.980

*DF=degree of freedom

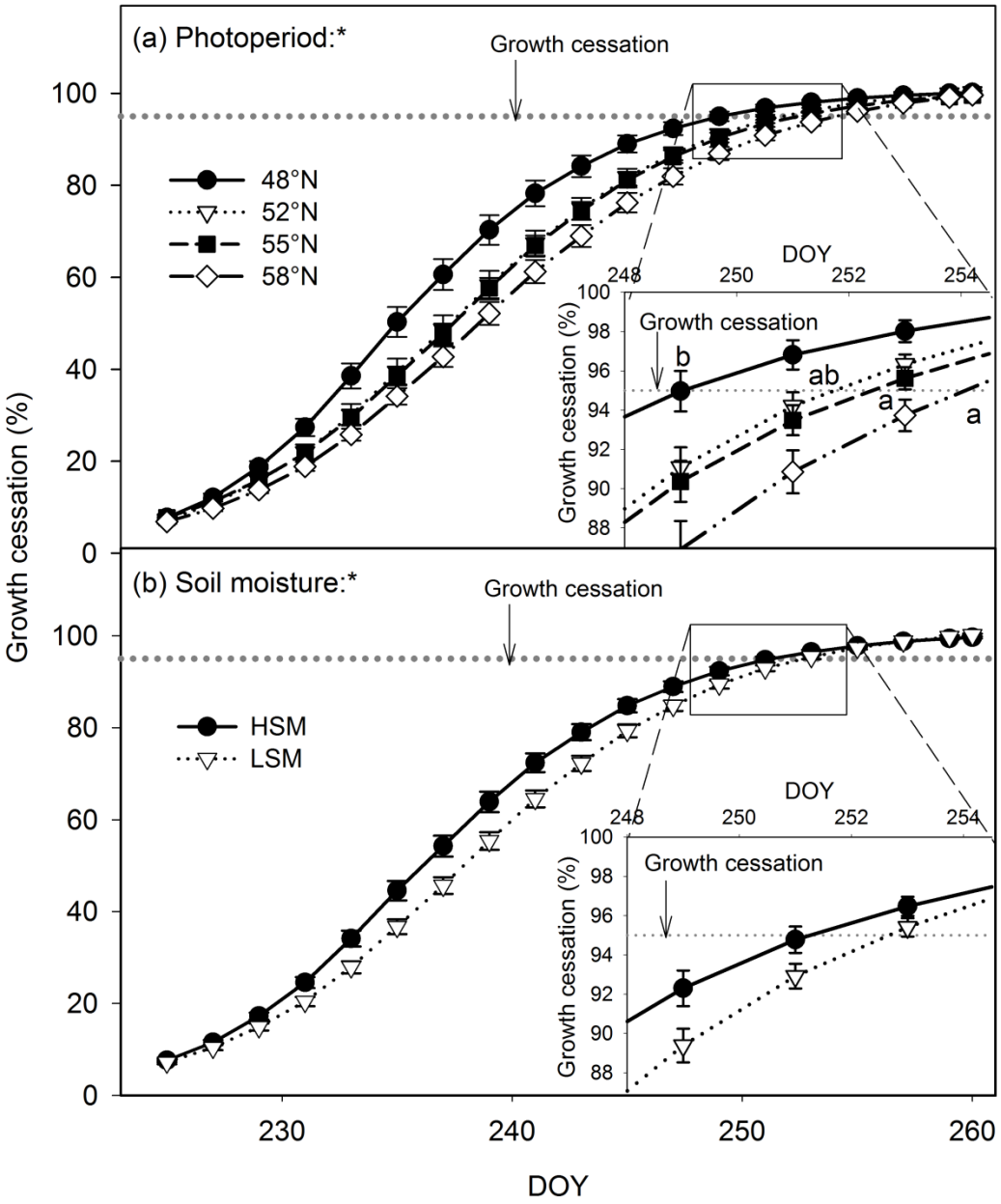

Figure 4.3 Mean (+SE) percentage of height growth increment. (a) photoperiod effect (n =24: three seedlings with two replications per treatment, two levels of [CO_2] and two levels of soil moisture, 3 x 2 x 2 x 2 = 24) and (b) represents the effect of soil moisture (n =48: three seedlings with two replications per treatment, two levels of [CO_2] and four levels of photoperiod, 3 x 2 x 2 x 4 = 48). The dashed line represents growth cessation. Growth cessation was defined as the date when 95% of height growth increment was achieved. The data were fitted with a 3-parameter sigmoidal model by using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). Different letters above the lines of (a) indicate significant differences under Fisher's Least Significant Difference post hoc test (p <0.05). The level of significance is shown (*p <0.05).

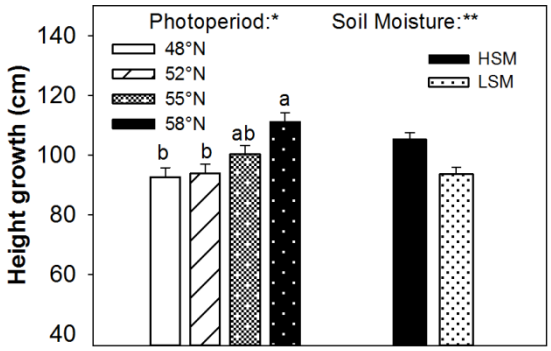

Figure 4.4 Total height by four photoperiods (at 48 (seed origin), 52, 55, and 58°N) (left) and two soil moisture levels (high and low) (right) in trembling aspen seedlings. Data are pooled across [CO_2] and soil moisture for (left) (n =24 (see Figure 4.3 for explanations), [CO_2] and photoperiod for (right) (n =48, (see Figure 4.3 for explanations). Different letters above the bars indicate significant differences under Fisher's Least Significant Difference post hoc test (p<0.05). The level of significance is shown (*p<0.05; **p<0.01).

Autumn bud phenology

In the first growing season, bud set was delayed in the combined treatments of EC with longer photoperiods (55˚N and 58˚N) (Figure 4.5a). In the second growing season, bud set was delayed by ~ nine and six days, respectively in the longer photoperiods of 55 and 58˚N (Figure 4.5b) and by ~20 days in LSM (Figure 4.5c).

Leaf senescence

The onset of leaf senescence in the first growing season was delayed by ~9 days and ~14 days by longer photoperiods of 55˚N and 58˚N latitude, respectively (p=0.054) as compared to the seed origin (Table 4.1, Figure 4.6a). However, the delaying effect on the completion of leaf senescence was much smaller (p =0.056, Figure 4.6a), leading to a faster rate and shorter duration of leaf senescence at longer photoperiods or higher latitudes, particularly at 58 ˚N (duration about 10 days shorter than at the seed origin). The photoperiod effects, however, disappeared in the second growing season (Table 4.1). [CO_2], soil moisture and their interaction

became significant on leaf senescence initiation and duration (Table 4.1). LSM significantly delayed the initiation but shortened the duration of leaf senescence, but the soil moisture effect was substantially reduced by EC (Table 4.1; Figure 4.6b). However, the completion of leaf senescence was only delayed by ~4 days in LSM.

Electrolyte leakage test

In the first growing season, LT_{50} generally became less negative with increases in photoperiod, (Table 4.1; Figure 4.7a). In the second growing season, however, the photoperiod effect on LT_{50} became statistically insignificant (Table 4.1). However, LSM substantially reduced the cold hardiness of the seedlings in the second growing season, as indicated by the much less negative LT_{50} (Table 4.1; Figure 4.7b).

Spring bud phenology

EC substantially delayed bud break in the spring, and the magnitude of the delay was greater in LSM (delayed by ~13 days in HSM, ~18 days in LSM) (Table 4.1; Figure 4.8a). LSM delayed bud break only under EC (Figure 4.8a). LSM slowed down leaf unfolding under AC but not under EC (Table 4.1; Figure 4.8b). However, EC expedited leaf unfolding in both moisture treatments and all photoperiod treatments (Table 4.1; Figure 4.8b).

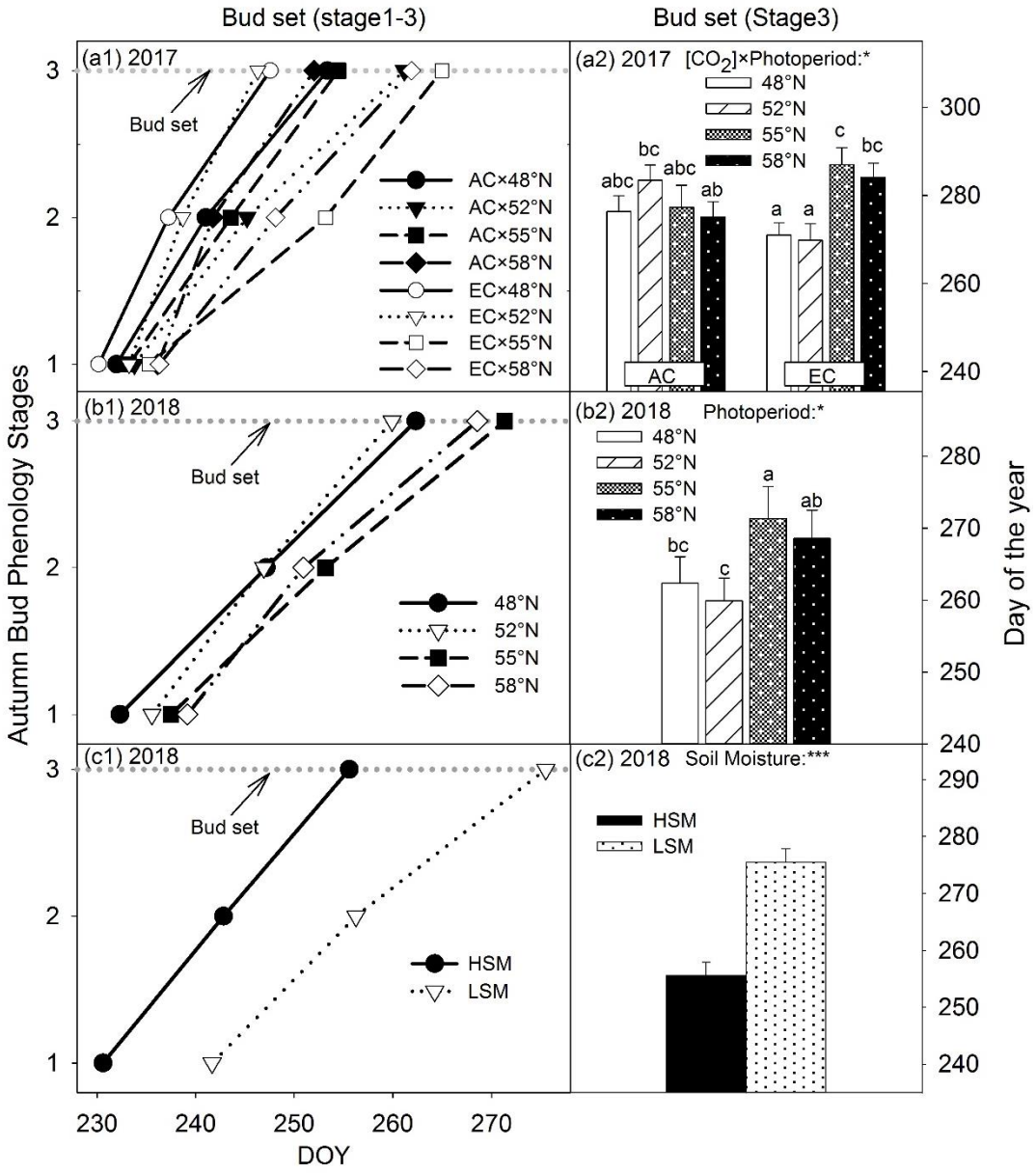

Figure 4.5 Mean day of the year at each autumn bud set stage in 2017 (a1) and 2018 (b1) and (c1) and mean (+SE) of the day of the year at stage3 (a2) 2017, and (b2) and (c2) 2018. (a1) and (a2) represent the interactive effect of [CO_2] and photoperiod effect (n =12: three seedlings with two replications per treatment, two levels of soil moisture, 3 x 2 x 2 = 12). (b1) and (b2) represents the effect of photoperiod (n =24 (see Figure 4.3 for explanations)). (c1) nad (c2) represent the soil moisture effect (n =24 (see Figure 4.3 for explanations)). Different letters above the lines of (a2) and (b2) indicate significant differences under Fisher's Least Significant Difference post hoc test (p <0.05). The level of significance is shown (*p <0.05; ***p <0.001).

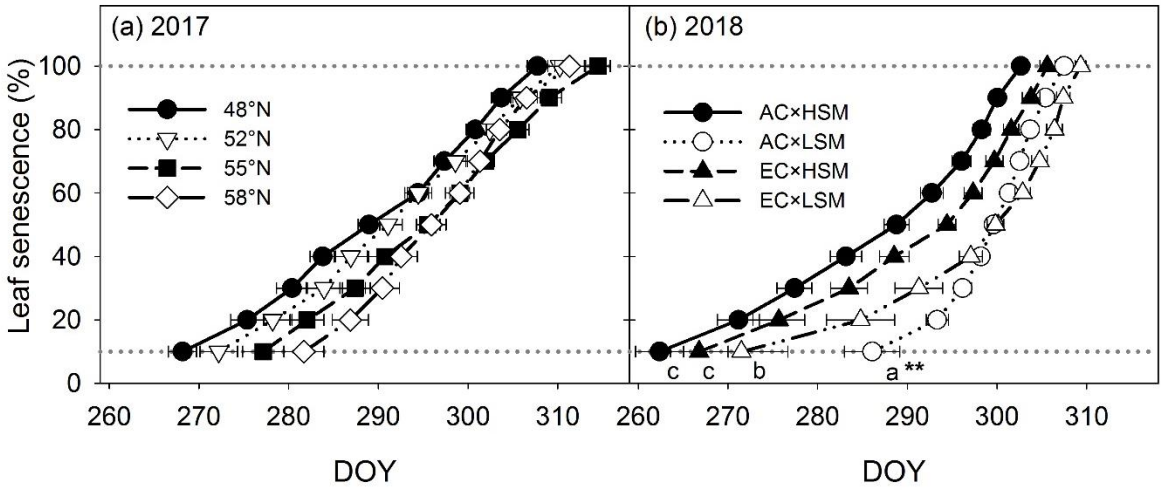

Figure 4.6 Mean (+SE) day of the year of leaf yellowing (%) in 2017(a), and 2018 (b). Data are pooled across [CO_2] and soil moisture (a) (n =24 (see Figure 4.3 for explanations)), and photoperiod for (b) (n =24: three seedlings with two replications per treatment, four levels of photoperiod, 3 x 2 x 4 = 24). Different letters in the bottom of (b) indicate significant differences under Fisher's Least Significant Difference post hoc test (p<0.05). The level of significance is shown (**p <0.01).

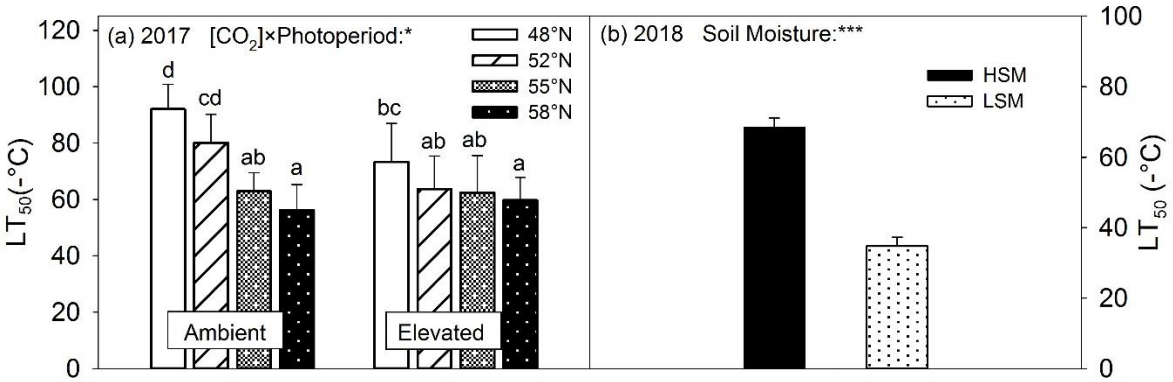

Figure 4.7 Mean (+SE) LT_{50} by an interactive effect of photoperiods (at 48 (seed origin), 52, 55, and 58°N) and [CO_2] (ambient and elevated) in 2017 (a) and the main effect of soil moisture (HSM and LSM) in 2018 (b) of trembling aspen seedlings. Data are pooled across [CO_2] and soil moisture for (a) (n =12, (see Figure 4.5 for explanations)), [CO_2] and photoperiod for (b) (n =48, (see Figure 4.3 for explanations)). Different letters above the bars represent significantly different means under Fisher's Least Significant Difference post hoc test (p <0.05). The level of significance is shown (*p <0.05; **; ***p <0.001) on the top.

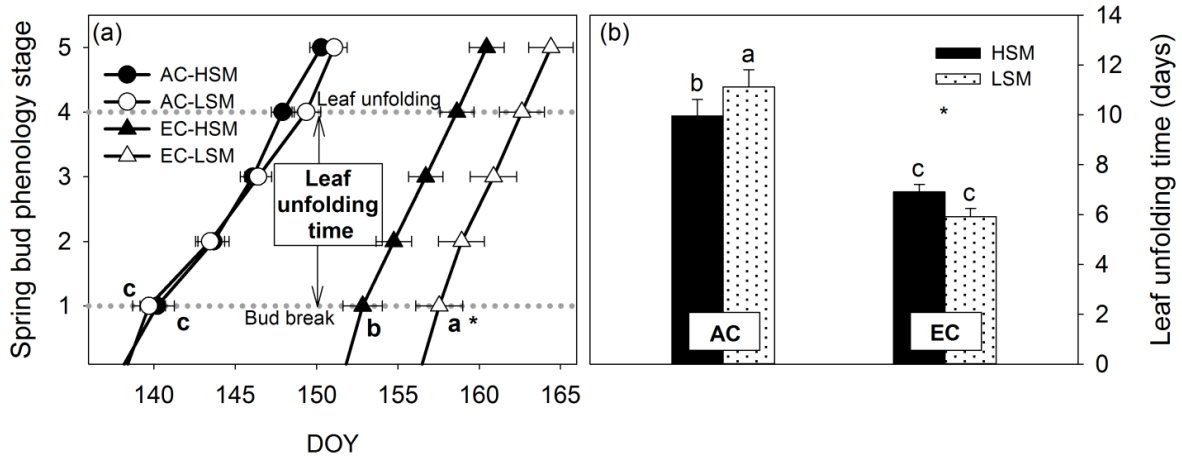

Figure 4.8 Mean (+SE) day of the year at each spring bud phenology stage of trembling aspen seedlings in 2018 (a). Figure (b) represents the interactive effect of [CO_2] and soil moisture on leaf unfolding time. Data are pooled across photoperiod and soil moisture for (a) (b) (n =24, (see Figure 4.6 for explanations)) because of the lack of significant effect (p >0.05). Different letters in the bottom (a) and above the bars (b) represent significantly different means under Fisher's Least Significant Difference post hoc test (p <0.05). Significance levels (as significant levels; *p <0.05) were indicated on the top.

4.5 Discussion

My results support the hypothesis that longer photoperiods increase growth but delay autumnal phenological events/processes. The two longest photoperiod regimes in this study delayed growth cessation in the fall by 5-6 days, bud set by 6-9 days, and the initiation of leaf senescence by 9-14 days, leading to an extended growing season and greater growth. These results suggest that the fall phenology of trembling aspen is probably triggered by a critical photoperiod and that the critical photoperiod is probably coded genetically which will not change when a population migrates or is moved to a new location with a different photoperiod regime. This conclusion agrees with the findings of Brissette & Barnes (1984), Frewen et al. (2000) and Howe et al. (1995). Other researchers have also concluded that photoperiod is the key environmental factor determining the initiation of the autumnal phenological processes in *Populus* spp. (Pauley and Perry 1954, Barr et al. 2004, Keskitalo et al. 2005, Fracheboud et al. 2009). *Salix pentandra*

(Junttila & Kaurin 1990) and *Betula pedula* (Viherä-Aarnio et al. 2005) also show delayed growth cessation in the fall when grown at higher latitudes than the seed origin. A long-term (10 years) field study has demonstrated that trembling aspen grows faster when it is grown at 7° north of the seed origin (Schreiber et al. (2013). It is interesting to note that in the present study, the delay in bud set under photoperiods at higher latitudes (55 and 58˚N) only occurred under elevated [CO_2] in the first growing season. This interactive effect may reflect the effect of elevated [CO_2] on the production and accumulation of carbohydrates (Ainsworth and Long 2005) and the effect of carbohydrate concentration on bud set (Ruttink et al. (2007). While the delay in autumn phenology and subsequent extension of the growing season promote growth when trees migrate to higher latitudes (Thomas and Vince-Prue 1997), such migration may also increase the risk of frost injuries if it also delays or jeopardize the proper development of cold hardiness (Howe, Saruul, Davis, & Chen, 2000). Indeed, longer photoperiods reduced cold hardiness in this study (significant only in the first growing season). Additionally, frost-damaged buds and reduced leaf nitrogen resorption due to delays in leaf senescence may prevent bud break in the following spring and make trees more susceptible to pest attacks (Ostry et al. 1989). Repeated damages will reduce the vigor and growth of trees (Zalasky 1976).

Surprisingly, low soil moisture delayed the onset of autumnal phenology and reduced cold hardiness in trembling aspen. Low soil moisture delayed the initiation of growth cessation but expedited the process of leaf senescence, leading to a shorter duration of the growth cessation process. It also delayed bud set by as much as 20 days. However, although the seedlings retained green leaves longer in the season in the low soil moisture treatment, the seedling height growth was decreased, and low soil moisture effect was counteracted by elevated [CO_2]. The delay in autumnal phenology in low soil moisture is in contradiction to my hypothesis and the results of

some previous studies. For instance, Holland et al. (2016) found that water stress treatment induces the onset of leaf senescence in *Quercus pubescens* about one month earlier in a greenhouse experiment. Similarly, water deficit, along with higher temperature, has resulted in earlier leaf senescence in *Betula pendula* (Juknys et al. 2012). However, My findings appear to be supported by several other studies (Vander Mijnsbrugge et al. 2016, Arend et al. 2016, Čehulić et al. 2019). The result that low soil moisture reduced the cold hardiness is also contrary to my hypothesis and other studies on the effect of water stress on cold hardiness. For instance, Kreyling et al. (2012) found increases in cold hardiness in *Pinus nigra* following summer drought. The decrease of cold hardiness in low soil moisture was possibly associated with the delay of phenological events by low soil moisture as discussed earlier. The effect of low soil moisture increased in the second growing season. It can be reasonably assumed that the water stress was severer in the second growing season since the water stress increase with tree size by increasing gravity and hydraulic resistance (Koch and Fredeen 2005). The lower cold hardiness (LT_{50} below -37 ºC) due to low soil moisture would increase freezing damage and tree mortality in extremely cold winter. A possible explanation for the delay in the present study could be the recovery process from severe water deficit during summer. Plants use various chemical signals (e.g., reactive oxygen species and plant hormones such as abscisic acid) to induce tolerance under water stress (Xu et al. 2010), which are also crucial for autumnal phenology induction (Woo et al. 2013). These chemicals decrease when water stress is reduced as a part of the recovery process, leading to a delay in induction of autumnal phenology (Xu et al. 2010, Vander Mijnsbrugge et al. 2016). Although my low soil moisture treatment continued during autumn, I postulate that along with temperature reduction from summer to autumn, the severity of water stress was reduced and the autumnal phenological process was interfered with this recovering

process, causing the observed delay.

The cold hardiness of aspen seedlings showed a decreasing trend with increasing photoperiod, and the photoperiod effect was reduced by elevated [CO_2]. The reduction in cold hardiness was accompanied by delays in leaf senescence, growth cessation, and bud set. The cold hardening process is initiated by short days or a combination of short days and cooler temperatures (Bigras et al. 2001), but the development of cold hardiness is complex and influenced by many factors, including the speed of acclimation after the first stage of cold hardiness occurs (Greer et al. 1989). Despite the changes in cold hardiness, aspen seedlings in all photoperiods and [CO_2] levels were sufficiently hardy (LT_{50} below -50 ºC) to withstand the cold winter in the Canadian boreal forest region (Black et al. 2005, Gusta et al. 2009). The influence of photoperiod on cold hardiness, however, disappeared in the second year measurement. The difference of the effect of photoperiod in each year may be in part due to the different age of trees (Lim et al. 2014). Especially seedling stage is the most vulnerable to cold injury (Howe et al., 2003).

Surprisingly, elevated [CO_2] delayed bud break but expedited leaf unfolding in the spring, and the effect was magnified by the low soil moisture treatment. The results of CO_2 effects on tree phenology in the literature vary with tree species and studies. Elevated [CO_2] do not affect bud break of *Populus* spp in some studies (e.g., *Populus trichocarpa* (Sigurdsson 2001) and *Populus* × *euramericana* (Calfapietra et al. 2003)) but delayed spring bud break of *Populus* clones in other research (Ceulemans et al. 1995). The delay I found was quite significant compared to the other studies. Delays in leafing out in the spring can have a negative impact on the competitiveness of pioneer tree species such as trembling (Lechowicz, 1984). However, it is not clear why the drought stress further aggravated the delay.

In summary, photoperiod regimes associated with a northward migration significantly affected autumnal phenological events/processes in trembling aspen, such as delays in growth cessation, bud set, and leaf senescence that led to longer growing season. The consequent increases in height growth may provide trembling aspen competitive advantages with other species. Height growth is recognized as an important trait for survival and frequently used as a measure of fitness (Ying and Yanchuk 2006), especially in the juvenile stage when a greater height will be advantageous in competition for light (Burns et al. 1990). Having a faster rate of height growth is crucial for the survival of pioneer species as trembling aspen (Ying and Yanchuk 2006). However, the delays in growth cessation and bud set can lead to delays in dormancy and cold hardiness development and expose trees to greater risks of frost injury. Moreover, other factors may constrain or offset the positive effect of longer photoperiod on growth. For instance, elevated [CO_2] delayed bud break in the spring, shortening the growing season. If northward migration is accompanied by reduced soil moisture as predicted for many parts of the boreal forest (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014), the interactions between elevated [CO_2], longer photoperiod, faster rate of its change and drier soil moisture can have detrimental effects on trees, particularly on frost resistance and frost damages. Therefore, changes in photoperiod, [CO_2] and soil moisture should be considered when planning latitudinal seed transfer and predicting natural migration of boreal trees in response to climate change.

4.6 Reference

Ainsworth EA, Long SP (2005) What have we learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol 165:351–372.

Arend M, Sever K, Pflug E, Gessler A, Schaub M (2016) Seasonal photosynthetic response of European beech to severe summer drought: Limitation, recovery and post-drought stimulation. Agric For Meteorol 220:83–89.

Arora R, Wisniewski ME, Scorza R (1992) Cold acclimation in genetically related (sibling) deciduous and evergreen peach (*Prunus persica* [L.] Batsch). Plant Physiol 99:1562–1568.

Barr AG, Black TA, Hogg EH, Kljun N, Morgenstern K, Nesic Z (2004) Inter-annual variability in the leaf area index of a boreal aspen-hazelnut forest in relation to net ecosystem production. Agric For Meteorol 126:237–255.

Bigras FJ, Ryyppö A, Lindström A, Stattin E (2001) Cold acclimation and deacclimation of shoots and roots of conifer seedlings. In: Bigras FJ, Colombo SJ (eds) Conifer cold hardiness. Springer Netherlands, pp 57–88.

Black TA, Gaumont-Guay D, Jassal RS, Amiro BD, Jarvis PG, Gower ST, Kelliher FM, Dunn A, Wofsy SC (2005) Measurement of CO_2 exchange between Boreal forest and the atmosphere. SEB Exp Biol Ser:151–185.

Boorse GC, Bosma TL, Ewers FW, Davis SD (1998) Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. Int J Plant Sci 159:513–521.

Brissette JC, Barnes BV (1984) Comparisons of phenology and growth of Michigan and western North American sources of *Populus tremuloides*. Can J For Res 14:789–793.

Burns RM, Honkala BH, Coordinators T (1990) Silvics of north america: volume 2. hardwoods. U S Dep Agric USDA For Serv Agric Handb 654

Calfapietra C, Gielen B, Sabatti M, De Angelis P, Miglietta F, Scarascia-Mugnozza G, Ceulemans R (2003) Do above-ground growth dynamics of poplar change with time under CO_2 enrichment? New Phytol 160:305–318.

Campbell RK, Sorensen FC (1973) Cold-acclimation in seedling douglas-fir related to phenology and provenance. Ecology 54:1148–1151.

Čehulić I, Sever K, Katičić Bogdan I, Jazbec A, Škvorc Ž, Bogdan S (2019) Drought impact on leaf phenology and spring frost susceptibility in a *Quercus robur* L. provenance trial. Forests 10:50.

Ceulemans R, Jiang XN, Shao BY (1995) Effects of elevated atmospheric CO_2 on growth, biomass production and nitrogen allocation of two *Populus* clones. J Biogeogr 22:261–268.

Chuine I (2010) Why does phenology drive species distribution? Philos Trans R Soc B Biol Sci 365:3149–3160.

Chuine I, Beaubien EG (2001) Phenology is a major determinant of tree species range. Ecol Lett 4:500–510.

Fracheboud Y, Luquez V, Björkén L, Sjödin A, Tuominen H, Jansson S (2009) The control of autumn senescence in European aspen. Plant Physiol 149:1982–1991.

Frewen BE, Chen THH, Howe GT, Davis J, Rohde A, Boerjan W, Bradshaw HD (2000) Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics 154:837–845.

Greer DH, Stanley CJ, Warrington IJ (1989) Photoperiod control of the initial phase of frost hardiness development in *Pinus radiata*. Plant Cell Environ 12:661–668.

Gunderson CA, Norby RJ, Wullschleger SD (1993) Foliar gas exchange responses of two deciduous hardwoods during 3 years of growth in elevated CO_2: no loss of photosynthetic enhancement. Plant Cell Environ 16:797–807.

Gusta LV, Wisniewski ME, Tanino KK (eds) (2009) Plant cold hardiness: from the laboratory to the field, First edition. CABI, Wallingford Oxfordshire, UK; Cambridge, MA.

Herrick JD, Thomas RB (2003) Leaf senescence and late-season net photosynthesis of sun and shade leaves of overstory sweetgum (*Liquidambar styraciflua*) grown in elevated and ambient carbon dioxide concentrations. Tree Physiol 23:109–118.

Hoch WA, Zeldin EL, McCown BH (2001) Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol 21:1–8.

Hogg EH, Bernier PY (2005) Climate change impacts on drought-prone forests in western Canada. For Chron 81:675–682.

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Can J For Res 38:1373–1384.

Hogg EH, Hurdle PA (1995) The aspen parkland in western Canada: A dry-climate analogue for the future boreal forest? Water Air Soil Pollut 82:391–400.

Holland V, Koller S, Lukas S, Brüggemann W (2016) Drought- and frost-induced accumulation of soluble carbohydrates during accelerated senescence in Quercus pubescens. Trees 30:215–226.

Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen TH (2003) From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can J Bot 81:1247–1266.

Howe GT, Hackett WP, Furnier GR, Klevorn RE (1995) Photoperiodic responses of a northern and southern ecotype of black cottonwood. Physiol Plant 93:695–708.

Howe GT, Saruul P, Davis J, Chen THH (2000) Quantitative genetics of bud phenology, frost damage, and winter survival in an F2 family of hybrid poplars. Theor Appl Genet 101:632–642.

Jach ME, Ceulemans R (1999) Effects of elevated atmospheric CO_2 on phenology, growth and crown structure of Scots pine (Pinus sylvestris) seedlings after two years of exposure in the field. Tree Physiol 19:289–300.

Jackson SD (2009) Plant responses to photoperiod. New Phytol 181:517–531.

Juknys R, Sujetoviene G, Zeimavicius K, Sveikauskaite I (2012) Comparison of climate warming induced changes in silver birch (*Betula pendula* Roth) and lime (*Tilia cordata* Mill.) phenology. Balt For 18:25–32.

Junttila O (1980) Effect of photoperiod and temperature on apical growth cessation in two ecotypes of *Salix* and *Betula*. Physiol Plant 48:347–352.

Junttila O (1982) The cessation of apical growth in latitudinal ecotypes and ecotype crosses of *Salix pentandra* L. J Exp Bot 33:1021–1029.

Junttila O, Kaurin Å (1990) Environmental control of cold acclimation in *Salix pentandra*. Scand J For Res 5:195–204.

Kaelke CM, Dawson JO (2005) The accretion of nonstructural carbohydrates changes seasonally in *Alnus incana* ssp. rugosa in accord with tissue type, growth, N allocation, and root hypoxia. Symbiosis Rehovot 39:61–66.

Keskitalo J, Bergquist G, Gardeström P, Jansson S (2005) A cellular timetable of autumn senescence. Plant Physiol 139:1635–1648.

Koch GW, Fredeen AL (2005) Transport challenges in tall trees. In: Holbrook NM, Zwieniecki MA (eds) Vascular transport in plants. Academic Press, Burlington, pp 437–456.

Kozlowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Bot Rev 68:270–334.

Kreyling J, Wiesenberg GLB, Thiel D, Wohlfart C, Huber G, Walter J, Jentsch A, Konnert M, Beierkuhnlein C (2012) Cold hardiness of *Pinus nigra* Arnold as influenced by geographic origin, warming, and extreme summer drought. Environ Exp Bot 78:99–108.

Leuzinger S, Zotz G, Asshoff R, Körner C (2005) Responses of deciduous forest trees to severe drought in Central Europe. Tree Physiol 25:641–650.

Li C, Junttila O, Ernstsen A, Heino P, Palva ET (2003) Photoperiodic control of growth, cold acclimation and dormancy development in silver birch (*Betula pendula*) ecotypes. Physiol Plant 117:206–212.

Lim C-C, Krebs SL, Arora R (2014) Cold hardiness increases with age in juvenile Rhododendron populations. Front Plant Sci 5.

MacLachlan IR, Wang T, Hamann A, Smets P, Aitken SN (2017) Selective breeding of lodgepole pine increases growth and maintains climatic adaptation. For Ecol Manag 391:404–416.

Marchetti SB, Worrall JJ, Eager T (2011) Secondary insects and diseases contribute to sudden aspen decline in southwestern Colorado, USA. Can J For Res 41:2315–2325.

McKenney DW, Pedlar JH, Lawrence K, Campbell K, Hutchinson MF (2007) Potential impacts of climate change on the distribution of North American trees. BioScience 57:939–948.

Moench RD (1999) Aspen seed collection. Nursery Information Series Colorado State Forest Service, Fort Collins CO.

Morin X, Améglio T, Ahas R, Kurz-Besson C, Lanta V, Lebourgeois F, Miglietta F, Chuine I (2007) Variation in cold hardiness and carbohydrate concentration from dormancy induction to bud burst among provenances of three European oak species. Tree Physiol 27:817–825.

Ostry ME, Wilson LF, McNabb HS (1989) A guide to insect, disease, and animal pests of poplars. United States Department of Agriculture.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., et al. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited By*R.K. Pachauri and L. Meyer. IPCC, Geneva, Switzerland.

Pauley SS, Perry TO (1954) Ecotypic variation of the photoperiodic response in *Populus*. J Arnold Arbor 35:167–188.

Peterson EB, Peterson NM (1992) Ecology, management and use of aspen and balsam poplar in the prairie provinces, Canada. Special Report 1.

Rapacz M (2002) Cold-deacclimation of oilseed rape (*Brassica napus* var. *oleifera*) in response to fluctuating temperatures and photoperiod. Ann Bot 89:543–549.

Regier N, Streb S, Zeeman SC, Frey B (2010) Seasonal changes in starch and sugar content of poplar (*Populus deltoides* × *nigra* cv. *Dorskamp*) and the impact of stem girdling on carbohydrate allocation to roots. Tree Physiol 30:979–987.

Riikonen J, Kets K, Darbah J, Oksanen E, Sober A, Vapaavuori E, Kubiske ME, Nelson N, Karnosky DF (2008) Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO_2 and O_3. Tree Physiol 28:243–254.

Ruttink T, Arend M, Morreel K, Storme V, Rombauts S, Fromm J, Bhalerao RP, Boerjan W, Rohde A (2007) A molecular timetable for apical bud formation and dormancy induction in poplar. Plant Cell 19:2370–2390.

Schreiber SG, Ding C, Hamann A, Hacke UG, Thomas BR, Brouard JS (2013) Frost hardiness vs. growth performance in trembling aspen: an experimental test of assisted migration. J Appl Ecol 50:939–949.

Sigurdsson BD (2001) Elevated [CO_2] and nutrient status modified leaf phenology and growth rhythm of young *Populus trichocarpa* trees in a 3-year field study. Trees 15:403–413.

Soolanayakanahally RY, Guy RD, Silim SN, Song M (2013) Timing of photoperiodic competency causes phenological mismatch in balsam poplar (*Populus balsamifera* L.). Plant Cell Environ 36:116–127.

Taylor G, Tallis MJ, Giardina CP, Percy KE, Miglietta F, Gupta PS, Gioli B, Calfapietra C, Gielen B, Kubiske ME, Scarascia-Mugnozza GE, Kets K, Long SP, Karnosky DF (2008) Future atmospheric CO_2 leads to delayed autumnal senescence. Glob Change Biol 14:264–275.

Thomas B, Vince-Prue D (1997) Photoperiodism in plants. Academic Press.

Tricker PJ, Calfapietra C, Kuzminsky E, Puleggi R, Ferris R, Nathoo M, Pleasants LJ, Alston V, Angelis PD, Taylor G (2004) Long-term acclimation of leaf production, development, longevity and quality following 3 yr exposure to free-air CO_2 enrichment during canopy closure in *Populus*. New Phytol 162:413–426.

Vander Mijnsbrugge K, Turcsán A, Maes J, Duchêne N, Meeus S, Steppe K, Steenackers M (2016) Repeated summer drought and re-watering during the first growing year of Oak (Quercus petraea) Delay autumn senescence and bud burst in the following spring. Front Plant Sci 7.

Viherä-Aarnio A, Häkkinen R, Partanen J, Luomajoki A, Koski V (2005) Effects of seed origin and sowing time on timing of height growth cessation of *Betula pendula* seedlings. Tree Physiol 25:101–108.

Way DA, Montgomery RA (2015) Photoperiod constraints on tree phenology, performance and migration in a warming world. Plant Cell Environ 38:1725–1736.

Weiser CJ (1970) Cold resistance and injury in woody plants: Knowledge of hardy plant adaptations to freezing stress may help us to reduce winter damage. Science 169:1269–1278.

Welling A, Kaikuranta P, Rinne P (1997) Photoperiodic induction of dormancy and freezing tolerance in *Betula pubescens*. Involvement of ABA and dehydrins. Physiol Plant 100:119–125.

Woo HR, Kim HJ, Nam HG, Lim PO (2013) Plant leaf senescence and death – regulation by multiple layers of control and implications for aging in general. J Cell Sci 126:4823–4833.

Worrall JJ, Rehfeldt GE, Hamann A, Hogg EH, Marchetti SB, Michaelian M, Gray LK (2013) Recent declines of *Populus tremuloides* in North America linked to climate. For Ecol Manag 299:35–51.

Xu Z, Zhou G, Shimizu H (2010) Plant responses to drought and rewatering. Plant Signal Behav 5:649–654.

Ying CC, Yanchuk AD (2006) The development of British Columbia's tree seed transfer guidelines: Purpose, concept, methodology, and implementation. For Ecol Manag 227:1–13.

Zalasky H (1976) Frost damage in poplar on the prairies. For Chron 52:61–64.

Zhang MIN, Willison JHM (1987) An improved conductivity method for the measurement of frost hardiness. Can J Bot 65:710–715.

5 Chapter 5: General discussions

5.1 Summary of the study

The objective of my thesis was to investigate the interactive effect of soil moisture, photoperiod, and [CO_2] on trembling aspen responses including physiology, morphology, and phenology in the context of the northward migration. In the face of rapid climate change predicted by Intergovernmental Panel on Climate Change (IPCC), trees must either adapt to the change or migrate to new locations or, in the worst-case scenario, go extinct (Aitken et al. 2008). McKenney et al. (2007) have assessed the potential impact of climate change on the distribution of approximately 130 tree species in North America and suggested an average northward migration of the climate habitat for these tree species of roughly 1000 km by 2100. Natural and/or human-assisted migration to higher latitudes will expose trees to different photoperiods from what they are adapted to (Howe et al. 2003), which would affect tree growth and survival. These photoperiod influences may have complex interactive effects with other predicted environmental factors, such as higher [CO_2] elevation and drier soil moisture. I strived to understand how soil moisture, photoperiod, and [CO_2] would influence the potential northward migration of trembling aspen in response to climate change. In this chapter, I summarize the key findings of each chapter, synthesize the results and discuss the limitations of the study.

Chapter 2: Height growth increase but cavitation resistance decreases in photoperiod at higher latitude under elevated [CO_2]

The results of Chapter 2 highlight the importance of incorporating the vulnerability to cavitation in predicting the northward migration of trembling aspen. I found that trembling aspen seedlings may have higher potential height growth and biomass productivity, at least at the juvenile stage,

when they migrate up to 10˚ north of their seed origin. However, drier soil conditions would offset height growth and total biomass productivity. Trembling aspen would become more vulnerable to drought stress because their resistance to xylem cavitation would decline with increasing photoperiod at higher latitudes under future [CO_2]. My results indicate that moving trembling aspen more than 7˚ northward could increase the risk of catastrophic hydraulic failure, resulting in more trembling aspen dieback or even tree mortality. Droughts are predicted to be frequent and more severe in the future in some parts of Canada (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). Therefore, any reduction in cavitation resistance at a higher latitude would hinder the northward migration of the species. The results indicate the existence of a possible threshold change in photoperiod beyond which the decline in the resistance xylem cavitation would cause catastrophic hydraulic failure. However, the study was conducted on young seedlings and the results may have limited implications to older trees in the field. Nevertheless, the seedling stage is the most important in the establishment of forest trees and seedlings are more vulnerable to environmental stresses (Grossnickle and Folk 1993, Close et al. 2005).

Chapter 3: The counterintuitive physiological responses to water stress indicate the critical and predominant role of morphological acclimation responding to low soil moisture

The results in Chapter 3 indicate that, apart from [CO_2] or photoperiod effects, the predicted drier soil conditions in many regions of North America (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014) might be a dominant limiting factor to the productivity of trembling aspen. A significant finding of this chapter is that morphological acclimation was the primary mechanism for trembling aspen in its response to low soil moisture. The increased root mass ratio reduced the level of moisture stress that the seedlings experienced internally because the

same amount of roots supported a smaller amount of leaves, resulting in an increase in P_n. The combination of higher P_n and increased biomass allocation to roots resulted in much higher photosynthetic water use efficiency for seedlings grown under the low soil moisture, particularly under the elevated [CO_2]. Further, the increase of photosynthesis in trembling aspen grown under low soil moisture was associated with an upregulation of photosynthetic apparatus as indicated by higher V_{cmax}, $J_{max,}$ and F_v/F_m. I also found that low soil moisture increased V_{cmax} and J_{max} under longer photoperiod, which indicate that longer photoperiod may improve acclamatory response to low soil moisture. However, under high soil moisture, longer photoperiod decreased *g*s, which suggest that the northward migration may induce water stress at the new locations.

Chapter 4: Projected drier soil constraints tree phenology associated with photoperiods at higher latitudes and future higher [CO_2]

The results of Chapter 4 reveal that both longer photoperiod and low soil moisture can delay the autumnal phenology of trembling aspen. Such delays under the longer photoperiod treatments increased height growth. Higher height growth is especially beneficial for pioneer tree species such as trembling aspen at the juvenile stage when competition for light is severe. However, the delay of autumnal phenology by longer photoperiods and low soil moisture was accompanied by lower cold hardiness, indicating that the trees may become more vulnerable from frost injury during the winter at higher latitudes (McKenney et al. 2007) or under the projected drier soil condition (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). Despite the decrease of cold hardiness due to longer photoperiods, the LT_{50} was still below -50 ^oC in all the treatment combinations and this level of cold hardiness may be sufficient for the trees to survive the cold temperatures during the winter in the boreal forest region of Canada (Black et al. 2005, Gusta et al. 2009). However, the low soil moisture treatment had a much bigger negative effect on cold

hardiness, with LT_{50} of only about -37˚, and thus trees growing on dry sites could suffer much greater damages from cold temperatures. Furthermore, [CO_2] elevation delayed bud break in the spring. Such a delay could potentially prevent seedlings from taking advantage of the better light conditions before taller trees leaf out and reduce the competitiveness of the species (Lechowicz 1984).

Limitations of the research

The study was conducted on young seedlings under controlled environmental conditions. Therefore the results may not be directly applicable to large trees grown in the field since eco-physiological responses vary with tree ontogeny and the environmental conditions in the field are highly variable. Sperry et al. (1991) have discovered that embolism occurs more often in older vessels than young ones since bordered pit membranes can deteriorate with age and older vessels have larger sized pit-membranes. Thus, cavitation resistance in larger trees is likely less than what is reported in this thesis. The seedling height growth was greater under longer photoperiods in both growing seasons in this study. However, the net effects were small and may not last (Merritt and Kohl 1982, 1983, Burdett and Yamamoto 1986). Therefore, the results should be interpreted with caution. Nonetheless, the growth and mortality play a key role in determining the fate of juvenile trees during natural regeneration (Harcombe 1987, King 1990).

Pot size can restrict root growth and function in plants. I used rather large pots in this study, particularly in the second growing season. However, because pots have walls on the side and limited depth and the growing media have very different physical and chemical properties, the below-ground growing environment is different from that at a natural forest site. The responses of trees grown in pots under controlled environmental conditions can be different from those in

the field (Curtis and Wang 1998, Norby et al. 1999). Thus, the results may not be directly applicable to trees grown in the field. Further studies in the field are warranted. However, the application of treatments is very challenging in the field.

5.2 Synthesis and conclusions

This study has provided valuable insights towards improving our understanding of the interactive effects of soil moisture, photoperiod and [CO_2] on trembling aspen (*Populus tremuloides* Michx.) in the context of climate change-induced northward migration. It is the first research investigating the interactive effects of soil moisture, photoperiod, and [CO_2] on trembling aspen. The findings of this study highlighted the importance to contemplate the interactive effects of soil moisture, photoperiod, and [CO_2] when assessing potential natural northward migration, planning human-assisted migration and projecting the future distribution of boreal forests. In addition to temperature, future distribution of boreal trees will depend on the interplay between multiple environmental and edaphic factors, thus parameters for future model predictions should consider these factors.

5.2.1 Synthesis of the study

Northward migration enhances tree growth, but delays phenological processes and increases the risk of frost injury coupled with low soil moisture and future [CO_2]

Northward migration induces higher height growth associated with different photoperiod regimes at the new locations (Chapter 2, 3 &4). The higher height growth is perhaps due to the lower photoperiod threshold of the seed collected from Thunder Bay (48.38°N, 89.25°W), which resulted in a longer growing season (Chapter 4). The longer growing season is a consequence of the delay in growth cessation and leaf senescence in photoperiods at higher latitude (Chapter 4)

and would have advantages for trembling aspen and would promote northward migration. Even small extensions of the growing season would increase net carbon fixation and overall tree growth. Height growth is recognized as one of the important traits for tree survival and frequently used as a measure of fitness (Ying and Yanchuk 2006). The height growth represents the tree's capacity for competition, which is one of the most important fitness components (Ying and Yanchuk 2006). Especially in the juvenile stage, the higher height growth can provide advantages when competing for resources such as light (Simard 1992). Particularly, for fast-growing species, including trembling aspen which is known as a shade-intolerance species (Burns et al. 1990), having higher height growth is crucial for survival at new locations. Simultaneously, however, by migrating trembling aspen toward the north, the risk of frost injury would increase by delaying bud set and leaf senescence (Chapter 4). Frost-damaged buds and frost damaged-leaves during leaf senescence would prevent a bud break in the following spring and cause trembling aspen to become susceptible to pest attacks (Ostry et al. 1989). If trembling aspen experiences these damages annually, its performance, such as vigor and growth, would be lowered (Zalasky 1976). Furthermore, elevated [CO_2] caused a substantial delay in spring bud break, which could be exacerbated by drier conditions (Chapter 4). Therefore, the potential benefit of using seeds from southern latitudes could be eventually offset by frost injury and delays in bud break associated with the northward migration and future [CO_2], leading to dieback or mortality at higher latitudes, which would potentially hinder trembling aspen's northward migration. The delay in bud break under elevated [CO_2] that could potentially lead lower growth performance reported here may conflict with the earlier findings of other studies which suggest that trembling aspen increases their growth resulting in competitive abilities enhancement under elevated [CO_2] (e.g., Lindroth et al. 1993).

The responses of trembling aspen to projected lower soil moisture and drought events in the future would be altered by photoperiod at higher latitudes and future [CO_2]

The theory that the positive effect of a northward migration on height growth will be constrained by severe water stress is supported by the findings of this thesis (Chapter 2, 3 &4) and that of Hogg et al. (2008). If trembling aspen moves northward by more than 7°, the results of this study suggest that it will become more vulnerable to cavitation, particularly under elevated [CO_2] (Chapter 2). The results suggest that there may be a threshold in terms of how far trembling aspen can be moved safely to higher latitudes and this threshold will likely be reduced if the frequency and severity of drought increase, as some studies have predicted in parts of the boreal forests (Pachauri, Allen, Barros, Broome, van Ypserle, et al. 2014). However, trembling aspen is less vulnerable to xylem cavitation than other poplar species (Lieffers et al. 2001). Since morphological acclimation is the primary mechanism in the response of trembling aspen to drought stress (Chapter 3) (e.g., Dang et al. 1997), trembling aspen may actually experience less severe drought stress physiologically and suffer less from hydraulic failure than other poplar species. Interestingly, the physiological and/or morphological acclimation to water stress can be stimulated by [CO_2] elevation and longer photoperiod (Chapter 3).

Past studies suggest that longer photoperiod and elevated [CO_2] may increase vessel/tracheid conduit diameter (Larson 1962, Kostiainen et al. 2014) and the total area of pits in the conduit, which is inversely related to cavitation resistance (Hacke and Sperry 2001, Tyree and Zimmermann 2002, Cai and Tyree 2010). Therefore, the lower cavitation resistance in longer photoperiods and elevated [CO_2] (Chapter 2) may be due to an increase in vessel diameters and total pit area. If this is true, the larger vessel diameter associated with longer photoperiod and elevated [CO_2] will make the species more vulnerable to freeze-thaw induced cavitation during

winter and spring (Davis et al. 1999, Venturas et al. 2017), which can lead to tree dieback (Cox and Malcolm 1997), and reduced growth and survival, further hindering the northward migration of the species. In fact, the connection between tree dieback and freeze-thaw events is reported by Zhu et al. (2000). Thus, further research should be undertaken to investigate the interactive effect of photoperiod and [CO_2] on xylem structure and xylem resistance to freeze-thaw induced cavitation.

5.3 Reference

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. Evol Appl 1:95–111.

Black TA, Gaumont-Guay D, Jassal RS, Amiro BD, Jarvis PG, Gower ST, Kelliher FM, Dunn A, Wofsy SC (2005) Measurement of CO_2 exchange between Boreal forest and the atmosphere. SEB Exp Biol Ser:151–185.

Burdett AN, Yamamoto S (1986) Growth rate and shoot: root allometry in *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus contorta* Dougl. Seedlings raised under two photoperiodic regimes. Scand J For Res 1:397–402.

Burns RM, Honkala BH, Coordinators T (1990) Silvics of north america: volume 2. hardwoods. U S Dep Agric USDA For Serv Agric Handb 654

Cai J, Tyree MT (2010) The impact of vessel size on vulnerability curves: data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx. Plant Cell Environ 33:1059–1069.

Close DC, Beadle CL, Brown PH (2005) The physiological basis of containerised tree seedling 'transplant shock': a review. Aust For 68:112–120.

Cox RM, Malcolm JW (1997) Effects of duration of a simulated winter thaw on dieback and xylem conductivity of *Betula papyrifera*. Tree Physiol 17:389–396.

Curtis PS, Wang X (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. Oecologia 113:299–313.

Dang Q-L, Margolis HA, Coyea MR, Sy M, Collatz GJ (1997) Regulation of branch-level gas exchange of boreal trees: roles of shoot water potential and vapor pressure difference. Tree Physiol 17:521–535.

Davis SD, Sperry JS, Hacke UG (1999) The relationship between xylem conduit diameter and cavitation caused by freezing. Am J Bot 86:1367–1372.

Grossnickle SC, Folk RS (1993) Stock quality assessment: Forecasting survival or performance on a reforestation site. Tree Plant Notes 44:113–121.

Gusta LV, Wisniewski ME, Tanino KK (eds) (2009) Plant cold hardiness: From the laboratory to the field, First edition. CABI, Wallingford Oxfordshire, UK; Cambridge, MA.

Hacke UG, Sperry JS (2001) Functional and ecological xylem anatomy. Perspect Plant Ecol Evol Syst 4:97–115.

Harcombe PA (1987) Tree Life Tables. BioScience 37:557–568.

Hogg EH, Brandt JP, Michaelian M (2008) Impacts of a regional drought on the productivity, dieback, and biomass of western Canadian aspen forests. Can J For Res 38:1373–1384.

Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen TH (2003) From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can J Bot 81:1247–1266.

King DA (1990) The adaptive significance of tree height. Am Nat 135:809–828.

Kostiainen K, Saranpää P, Lundqvist S-O, Kubiske ME, Vapaavuori E (2014) Wood properties of *Populus* and *Betula* in long-term exposure to elevated CO_2 and O_3. Plant Cell Environ 37:1452–1463.

Larson PR (1962) The indirect effect of photoperiod on tracheid diameter in *Pinus resinosa*. Am J Bot 49:132–137.

Lechowicz MJ (1984) Why do temperate deciduous trees leaf out at different times? Adaptation and Ecology of Forest Communities. Am Nat 124:821–842.

Lieffers VJ, Landhausser SM, Hogg EH (2001) Is the wide distribution of aspen a result of its stress tolerance? Shepperd Wayne Binkley Dan Bartos Dale Stohlgren Thomas J Eskew Lane G Comps Sustain Aspen West Landsc Symp Proc 13-15 June 2000 Gd Junction CO Proc RMRS-P-18 Fort Collins CO US Dep Agric For Serv Rocky Mt Res Stn P 311-324 18:311–324.

Lindroth RL, Kinney KK, Platz CL (1993) Responses of diciduous trees to elevated atmospheric CO_2: Productivity, phytochemistry, and insect performance. Ecology 74:763.

McKenney DW, Pedlar JH, Lawrence K, Campbell K, Hutchinson MF (2007) Potential impacts of climate change on the distribution of North American trees. BioScience 57:939–948.

Merritt RH, Kohl Jr HC (1982) Effect of root temperature and photoperiod on growth and crop productivity efficiency of *petunia* [Alternate heat sources, use of energy]. J-Am Soc Hortic Sci USA

Merritt RH, Kohl Jr HC (1983) Crop productivity efficiency of petunias in the greenhouse. J Am Soc Hortic Sci

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22:683–714.

Ostry ME, Wilson LF, McNabb HS (1989) A guide to insect, disease, and animal pests of poplars. United States Department of Agriculture.

Pachauri, R.K., Allen, M.R., Barros, V.R., Broome, J., Cramer, W., Christ, R., et al. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *Edited By*R.K. Pachauri and L. Meyer. IPCC, Geneva, Switzerland.

Simard S (1992) Ecology and management of paper birch and black cottonwood in southern British Columbia. B.C. Ministry of Forests, Victoria, B.C.

Sperry JS, Perry AH, Sullivan JEM (1991) Pit membrane degradation and air-embolism formation in ageing xylem vessels of *Populus tremuloides* Michx. J Exp Bot 42:1399–1406.

Tyree MT, Zimmermann MH (2002) Xylem structure and the ascent of sap, 2nd edn. Springer-Verlag, Berlin Heidelberg.

Venturas MD, Sperry JS, Hacke UG (2017) Plant xylem hydraulics: What we understand, current research, and future challenges. J Integr Plant Biol 59:356–389.

Ying CC, Yanchuk AD (2006) The development of British Columbia's tree seed transfer guidelines: Purpose, concept, methodology, and implementation. For Ecol Manag 227:1–13.

Zalasky H (1976) Frost damage in poplar on the Prairies. For Chron 52:61–64.

Zhu XB, Cox RM, Arp PA (2000) Effects of xylem cavitation and freezing injury on dieback of yellow birch (*Betula alleghaniensis*) in relation to a simulated winter thaw. Tree Physiol 20:541–547.